STUDY OF MINERAL BONE DISEASE AND CARDIOVASCULAR MORBIDITY IN RELATION WITH FIBROBLAST GROWTH FACTOR 23 IN CHRONIC KIDNEY DISEASE PATIENT PRESENTING TO SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES

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M.D. GENERAL MEDICINE

BRANCH I

MAY 2018

CERTIFICATE

This is to certify that this dissertation entitled "**Study of Mineral Bone Disease and Cardiovascular Morbidity in Relation with Fibroblast Growth Factor 23 in Chronic Kidney Disease Patient Presenting to Sree Mookambika Institute of Medical Sciences"** is a bonafide record of the work done by **Dr. Rishabh Gupta** during the period 2016-2019. This has been submitted in the partial fulfillment of the award of **M.D. Degree in General Medicine [Branch-I]** by the Tamilnadu Dr. MGR Medical University Chennai.

Dr. S. Thilagar
[Guide]
Professor
Department of General Medicine
Sree Mookambika Institute of
Medical Sciences [SMIMS]
Kulasekharam, K.K District,
Tamil Nadu -629161

Dr. Beena Unnikrishnan
[Co-Guide]
Associate Professor,
Department of Nephrology,
Sree Mookambika Institute of
Medical Sciences [SMIMS]
Kulasekharam, K.K District,
Tamil Nadu -629161

Dr. Rema V. Nair, M.D., D.G.O.,
Director
Sree Mookambika Institute of
Medical Sciences [SMIMS]
Kulasekharam, K.K District,
Tamil Nadu - 629 161

Dr. J. Kaniraj Peter, M.D.,
Professor and HOD,
Department of General Medicine,
Sree Mookambika Institute of
Medical Sciences [SMIMS]
Kulasekharam, K.K District,
Tamil Nadu - 629161

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DECLARATION

In the following pages is presented a consolidated report of the study **"Study of Mineral Bone Disease and Cardiovascular Morbidity in Relation with Fibroblast Growth Factor 23 in Chronic Kidney Disease Patient Presenting to Sree Mookambika Institute of Medical Sciences"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. S. Thilagar**, Professor, Department of General Medicine, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Tamilnadu.

Dr. Rishabh Gupta

Junior Resident
Department of General Medicine,
Sree Mookambika Institute of Medical Sciences,
Kulasekharam, Kanyakumari District.

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LIST OF ABBREVIATIONS

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
Calcitriol	1α,25-Dihydroxycholecalciferol
25(OH)D	25-hydroxyvitamin D
Calcidiol	25-hydroxychoelcalciferol
cholecalciferol	vitamin D
Cyp27b1	1α-hydroxylase
Cyp24a1	24-hydroxylase
ADHR	Autosomal Dominant Hypophosphatemic Rickets
AKI	Acute Kidney Injury
ARHR1 and 2	Autosomal Recessive Hypophosphatemic Rickets, Type 1 and 2
CaSR	Calcium-sensing receptor
CKD-MBD CKD	Mineral and Bone Disorder
cKL	Shedded full-length Klotho
CYP24A1	1,25-dihydroxyvitamin D 24-hydroxylase
CYP27B1	25-hydroxyvitamin D 1-alpha-hydroxylase
ESRD	End Stage Renal Disease

FGF	Fibroblast growth factor
FGF23	Fibroblast growth factor-23
FGFR	Fibroblast growth factor receptor
GALNT3	Polypeptide N-acetylgalactosaminyltransferase 3
GFR	Glomerular Filtration Rate
LVH	Left ventricular hypertrophy
PTH	Parathyroid hormone
TRPV5	Transient receptor potentialcation channel subfamily v member 5
VDR	Vitamin D receptor
XLH	x linked hypophosphatemia

ABSTRACT

ABSTRACT

Background:

Chronic kidney disease is a progressive disorder with involvement of various organs. CKD MBD is a systemic disorder of mineral and bone metabolism which is manifested by either one or a combination of abnormalities of calcium, phosphorous, PTH or Vit D metabolism. This results in changes in bone mineralisation or growth and other complications like vascular or other soft tissue calcification. The hormone fibroblast growth factor (FGF23) is derived from bone and its important function in controlling phosphorous levels. Elevation of serum levels of FGF23 is one of the possible causes of left ventricular hypertrophy and diastolic dysfunction. This leads to various morbidities and early mortalities in these patients. This study was done to observe calcium, phosphorous, iPTH and especially FGF23 and to note any role of FGF23 in causing cardiac involvement in these patients.

Methods:

Ethical committee clearance was obtained. After taking **informed** consent, 50 patients were enrolled in the study between September 2017 and February 2018.All subjects were in the group 20-70 years. Cardiac systolic function was assessed by echo. Mineral bone disorder was assessed by Serum FGF23, calcium, phosphorous, parathyroid hormone, in all patient. The data was analysed by SPSS VERSION 20.0

Results:

In our study found that among 50 study participants 88 percent patients had hyperphosphataemia, 64 percent had hypocalcemia, 46 percent had elevated parathormone and 74 percent had elevated FGF23. All the patients had biochemical evidence of mineral bone disorder. In them 20 percent had left ventricular hypertrophy, and left ventricular diastolic dysfunction. Reduced ejection was noted in 10 percent of patients. Positive correlation was noted with level of phosphorous and FGF23.The left ventricular hypertrophy was also noted in those with elevated FGF23 and it was statistically significant. Statistically there was no correlation with the level of calcium, ipth or reduced ejection fraction in relation to FGF23.

Conclusion:

CKD MBD is common in patients with ckd especially stage 5. They are also prone for cardiovascular manifestation. FGF23 levels were high in those with elevated phosphorous, left ventricular hypertrophy and left ventricular dysfunction. Levels of Calcium, ipth and ejection fraction did not correlate with FGF23. Larger population in the study would have helped in actual identification of these patients and also helped in the knowing the predominant role of FGF23.

Keyword: CKD, ckd mbd, FGF23, cardiovascular involvement

INTRODUCTION

INTRODUCTION

Chronic kidney disease (CKD) is characterized by slow and progressive loss of kidney function over years. Most of the time it goes undetected till severe irreparable damage has occurred. CKD is a worldwide public health problem. There is rising incidence and prevalence of patients with kidney failure which is usually associated with poor outcomes. The cost of treatment of this disease is very expensive.

CKD is prevalent in elderly population. Over the age of 65 years, about 30 percent of patients have a stable kidney disease.[1] while young patients are found to have early progressive loss of renal function.

Whatever the underlying etiology, once the loss of nephrons and reduction of functional renal mass reaches a certain point. The remaining nephrons begin a process of irreversible sclerosis that leads to a progressive reduction in the glomerular filtration rate (GFR).[2.]

CKD has been staged by KDIGO (Kidney Disease: Improving Global Outcome) 2012 according to GFR and albuminuria. This essentially helps to treat and control the disease according at various stages.

By CKD stages 3 and 4, changes in the body occurs affecting the bone and mineral metabolism. This syndrome has been named 'CKD-mineral bone disorder' (CKD-MBD).[1] The prevalence of various mineral bone disease abnormalities were 70% hyperphosphatemia, 85% hyperparathyroidism, and 100% low levels of

25(OH)D among the patients. But the earliest histological abnormalities of bone in CKD-MBD are seen after a relatively mild reduction in GFR in CKD stage l2.[3] By CKD stage 5, skeletal abnormalities are found in all patients. It is one of the dreaded complication and if not treated in time can lead to increased morbidity and mortality.

Disorders of bone and mineral homeostasis are also recognized to play an important role in cardiovascular complication of CKD. The current understanding of the initial mechanisms involved in the pathogenesis of CKD-MBD is focused on very early rise in the skeletal hormone, Fibroblast Growth Factor 23 (FGF23), as a sign of disturbed skeletal function, loss of skeletal anabolism, hyperphosphatemia, reduced calcitriol and secondary hyperparathyroidism.

In the clinical practice, we should monitor calcium, phosphorous, vitamin D level, parathormone and FGF23 status of our dialysis. This prevents further complications. The advances in the basic science of these serum factors and their interactions and changes in CKD Stage 5 are a source of great scientific interest. Understanding the changes in the levels of mentioned parameters will go a long way to improve the health and decrease the morbidity and mortality associated with it.

In this dissertation, we are doing a hospital based cross sectional study to find out the status of mineral bone disease in CKD patients in relation to FGF 23, Ca2+, phosphorus and intact Parathormone (iPTH) in patients in SMIMS, and to identify any cardiovascular morbidity in them.

AIMS & OBJECTIVES

AIM AND OBJECTIVES

- To find out the status of mineral bone disease in CKD patients irrespective of CKD stages in relation to FGF 23, Ca2+, phosphorus and intact parathormone (iPTH).
- To correlate with LV function with the help of echocardiographic findings

REVIEW OF LITERATURE

REVIEW OF LITERATURE

CHRONIC KIDNEY DISEASE (CKD)

CKD is a major health problem affecting 5-10% of the world population.[4] The main causes of impaired kidney function are diabetic nephropathy, hypertension, polycystic kidney disease (PKD) and some inflammatory and systemic disorders.

Glomerular filtration rate (GFR) is the measure of kidney function. It is used to determine the stage of kidney disease. The measurement of endogenous creatinine in blood can help to measure the estimated glomerular filtration rate (GFR).

CKD is defined as impaired kidney function or GFR <60 mL/min/1.73 m2 for more than 3 months. All individuals with GFR <60 mL/min/1.73 m2 for 3 months are classified as having CKD, irrespective of the presence or absence of kidney damage. This is associated with a complications like hypertension, hypocalcaemia, hyperphosphatemia and low hemoglobin and albumin levels.

CKD staging has been defined according to the level of GFR and albuminuria.

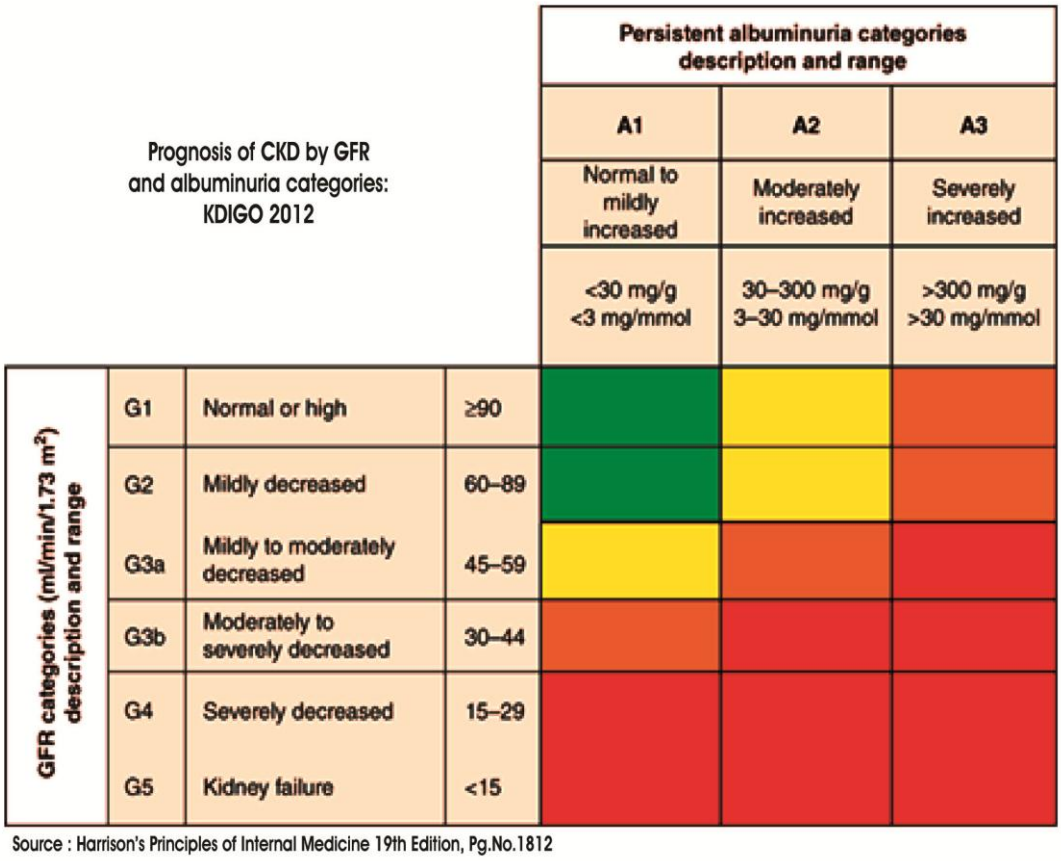

Fig. 1[5] Classification of CKD [KDIGO 2012]

CKD-Mineral and Bone Disorder (CKD-MBD)

Various epidemiological studies show a strong association between CKD related disturbances in mineral metabolism to bone abnormalities, cardiovascular disease (CVD) and overall mortality. All these myriads of features are aggregately called as CKD-mineral and bone disorder.

KDIGO 2012 has given the definition of CKD-MBD as a combination of

1. Abnormalities in calcium, phosphate, PTH and vitamin D metabolism
2. Abnormalities in bone metabolism and
3. Vascular or other soft tissue calcifications (Fig. 4).[6]

RENAL OSTEODYSTROPHY

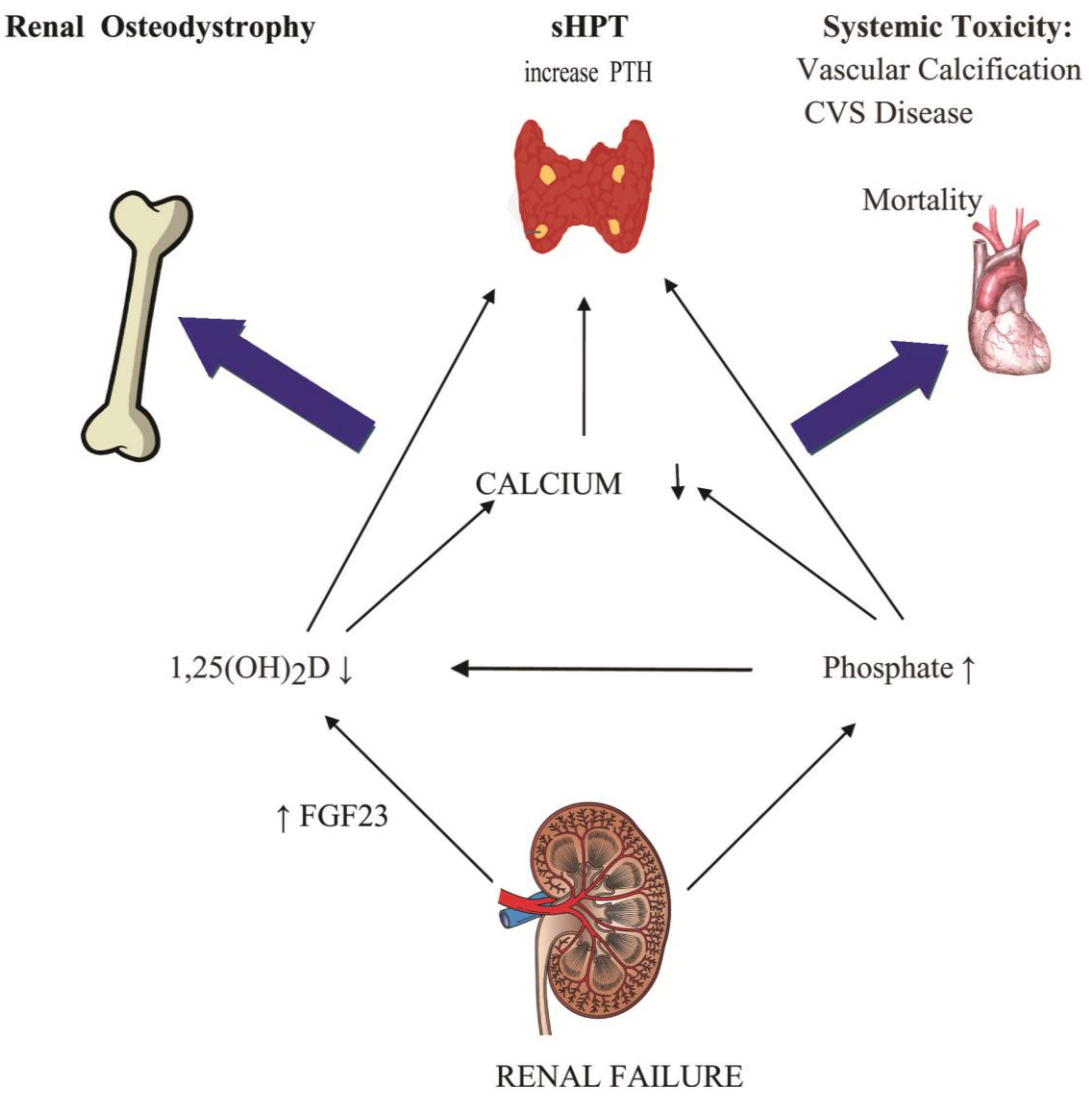

Fig. 2 Development CKDMBD[7,8]

METABOLISM OF PHOSPHATE

Phosphorous is an integral molecule in DNA and RNA. It is also an important constituent of cell membranes and intracellular organelles, and acts as the substrate for kinase and phosphatase regulation of intracellular signaling.[9] Phosphorous which is a nutrient essential for many biological processes including

skeletal mineralization and energy provision in the form of ATP is the 6th most common element in the body. Phosphorous in the blood does not bind to proteins and exists in forms like H2PO4-, HPO4-2 and PO4-3.

Hence, circulating phosphorous is often denoted as inorganic phosphate or phosphate. Human body contains about 500-700 g of phosphorus. 85% of which is hydroxyapatite crystals of the skeleton together with Ca2+, 15% is located in the cellular compartments and less than 1% of the total body phosphorous is located in the extracellular fluids.

Normal serum phosphate concentration is 2.2 – 4.5 mg/dL. This is maintained by the intestine, kidneys, bone and parathyroid gland and they are acting together.

HOMEOSTASIS OF PHOSPHATE

70% of dietary intake of phosphorus is absorbed from the intestinal lumen (Fig1). Average dietary intake of phosphorous is 1200 mg / day. Intestinal absorption of phosphorous is directly proportional to dietary intake and is regulated through NPT2b [sodium-dependent phosphate co-transporter type 2 b] by active transport. Daily exchange of about 150- 300 mg phosphate occurs between bone and extra-cellular fluid.

Between 60% and 70% of dietary phosphate is absorbed by the gastrointestinal tract in all intestinal segments. Phosphate absorption depends on both passive transport related to the concentration in the intestinal lumen (e.g., increased after a meal) and active transport stimulated by 1,25(OH)2D (calcitriol), the active metabolite of vitamin D. Passive absorption dependent on luminal

phosphate concentration accounts for the majority of the phosphate absorbed. Passive absorption occurs via epithelial brush border sodium phosphate co-transporter (Npt2b) using energy from the basolateral sodium–potassium ATPase transporter. The Npt2b sits in the terminal web just below the brush border in "ready to use" vesicles that are then transported to the brush border in response to acute and chronic changes in phosphate concentration.[9,10]

Medications or foods that bind intestinal phosphate (e.g., antacids, phosphate binders, Calcium) can decrease the net amount of phosphate absorbed by decreasing the free phosphate for absorption. Calcitriol can upregulate the sodium−phosphate co-transporter and actively increase phosphate absorption. However, in contrast to calcium, the active Vitamin D- mediated absorption of phosphate is a minor component of total absorption, supported by data that there is near normal intestinal absorption of phosphate in the absence of vitamin D.

The kidneys are responsible for maintaining phosphate balance by excreting the net amount of phosphate that is absorbed (Fig 54-1). Most inorganic phosphate is freely filtered by the glomerulus. Approximately 70% to 80% of the filtered load of phosphate is reabsorbed in the proximal tubule. The remaining 20% to 30% is reabsorbed in the distal tubule. Factors that increase phosphate excretion are increased plasma Pi concentration, PTH, metabolic acidosis and FGF23.

Majority of this regulation occurs in the proximal tubule via the sodium–phosphate co-transporter Npt2a. Similar to the intestine, the Npt2a rests in the terminal web and can be acutely moved to the brush border in the presence of acute or chronic Pi depletion. Alternatively, after a Pi load or in the presence of PTH or FGF23, the exchanger is removed from the brush border and catabolized.[11,12]

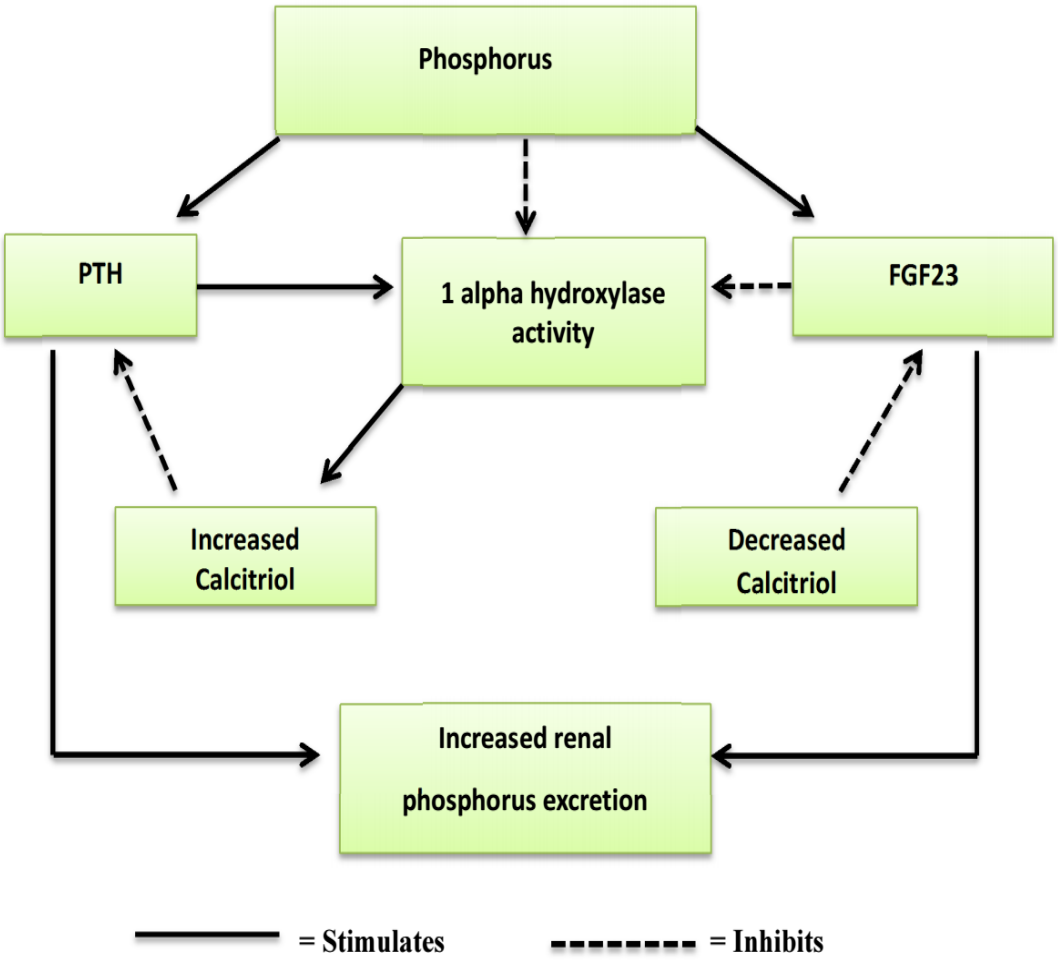

Fig 3 [9, 10] Phosphorous Regulation

This shows the regulation of phosphorous. When the phosphate excretion is decreased in our body and the levels are increased, it will stimulate PTH [parathyroid hormone] and FGF 23 [fibroblast growth factor 23] levels. Increase in PTH and FGF-23 will help to increase the phosphate excretion in urine. But they differ in action over 1 alpha hydrolase activity. PTH increases its activity whereas FGF-23 will inhibit its activity. By stimulating 1 alpha hydrolase activity it will increase the production vitamin D3, which in turn have negative feedback on parathyroid gland to decrease the production of PTH. But FGF-23 have a opposite

effect, it will inhibit 1 alpha hydroxylase activity thereby decreasing the levels of vitamin D3. Decreased levels of vitamin D3 [calcitriol] will in turn further stimulate the production of FGF-23.

Phosphorous Abnormalities in CKD[13]

The ability of the kidneys to control phosphate becomes impaired at a GFR of approximately 50 to 60 mL/min. Frank hyperphosphatemia is observed in most persons after the GFR is less than 20 to 25 mL/min. The maintenance of normal levels of phosphate when the GFR is between 30 and 50 mL/min has been thought to occur at the expense of continued increase in PTH and FGF23 secretion. Studies in humans have demonstrated that an oral load of phosphate increased PTH. However, it also decreased ionized calcium, possibly contributing to the increase in PTH.[13]

However, subsequent human studies controlled for changes in calcium and still found that phosphate loading increased PTH, and conversely, phosphate restriction inhibited the rise in PTH. Recent studies in humans also support that phosphate retention occurs earlier than had been previously appreciated. [13]

Although phosphate levels are maintained in the "normal" range in patients with CKD stages 3 and 4 (GFR, 30-60 and 15-30 mL/min), there is a gradual increase in the serum concentration, with progressive CKD indicating that a new "steady state" of slightly higher serum phosphate and increased PTH is reached. In addition, although there is a progressive increase in the fractional excretion of phosphate, there is a net decrease in phosphate excretion with progressive CKD[13]

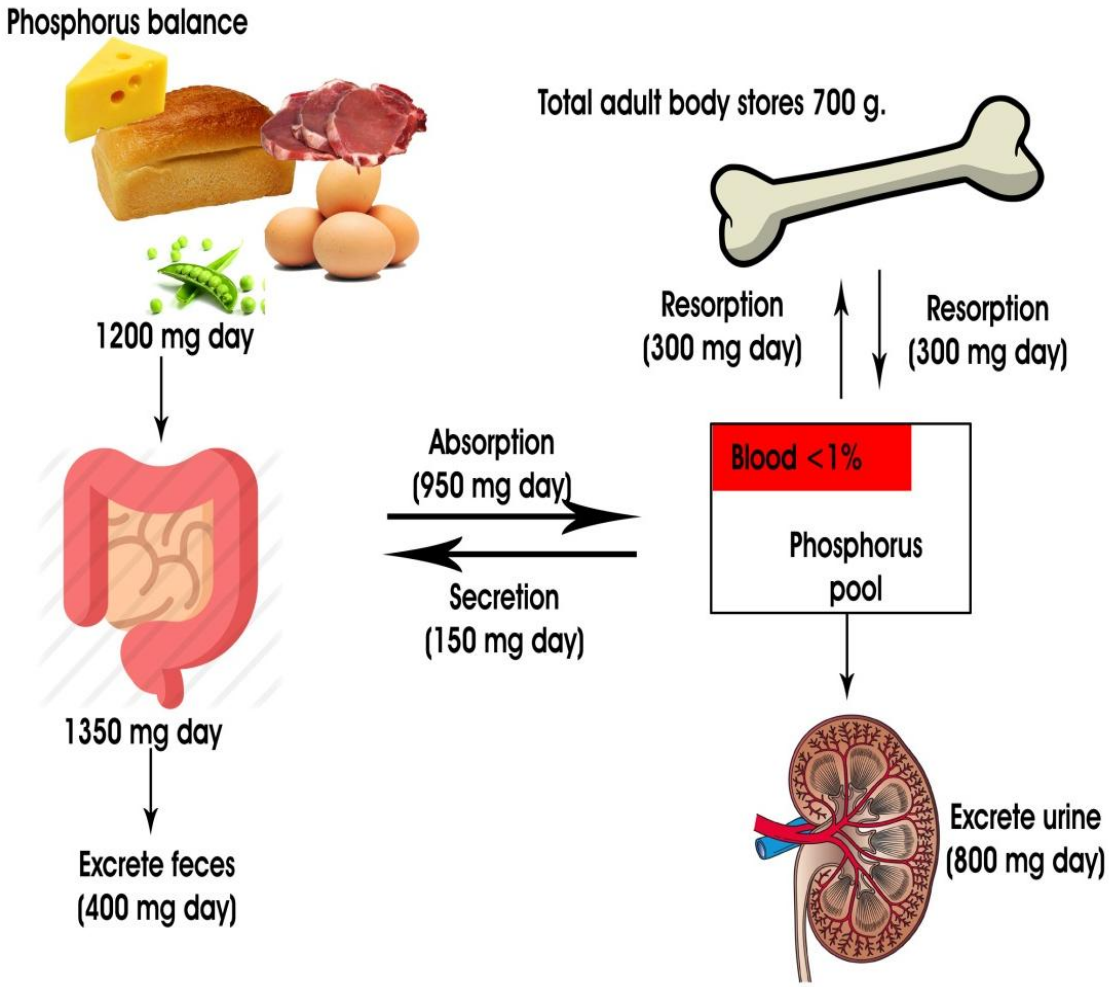

Fig. 4[14] Phosphorus Balance In Normal Physiology

Calcium metabolism:

Serum calcium concentrations are normally tightly controlled within a narrow range, usually 8.5 to 10.5 mg/dL (2.1–2.6 mmol/L). However, the serum calcium concentration is a poor reflection of overall total body calcium because serum levels are less than 1% of total body calcium. The remainder of total body calcium is stored in bone. Ionized calcium is physiologically active, but the non-ionized calcium is bound to albumin or anions such as citrate, bicarbonate, and Pi. In the presence of hypoalbuminemia, there is a relative increase in the ionized calcium relative to the total calcium. Therefore, total serum calcium may underestimate the physiologically active (ionized) serum calcium.[13]

However, a study in CKD stages 3 to 5 (non dialysis) patients found that total calcium or albumin-corrected calcium failed to correctly classify 20% of patients as either hypocalcaemic or hypercalcemic documented by ionized calcium. Serum levels of ionized calcium are maintained in the normal range by inducing increases in the secretion of PTH. PTH acts to increase bone reabsorption, increase renal calcium reabsorption, and increases the conversion of 25(OH)D to 1,25(OH)2D in the kidney, thereby increasing gastrointestinal calcium absorption. In individuals with CKD, the ability to maintain normal homeostasis, including a normal serum ionized calcium level and appropriate calcium balance for age is lost[13]

Early studies in patients with markedly advanced CKD not yet on dialysis showed negative calcium balance, although these patients were generally not taking vitamin D or calcium-containing phosphate binders or calcium supplements. Patients on dialysis frequently have significant intake of calcium from calcium-containing phosphate binders. [13]

In patients with CKD, approximately 18% to 20% of calcium is absorbed from the intestine; therefore, at 2000 mg/day in total elemental calcium intake (diet plus binder), the net intake is 400 mg/ day. On hemodialysis days, this figure may be altered up or down by approximately 50 mg/day depending on the dialysate flux, which in turn depends on the patient's serum calcium and dialysate calcium concentration and the type of dialysis.

The excretion of calcium in stool and sweat ranges from 150 to 250 mg/day, and if patients have residual urine output, the excretion rate may increase by 50 to 100 mg/day. Thus, with 400 mg net absorbed calcium, most patients will still be in positive calcium balance when taking 2000 mg/day of total elevated calcium if they are also taking calcitriol or other vitamin D analogues.. If the bone were remodeling normally, the calcium would be deposited there; however, normal bone is not common in dialysis patients.

If no calcium containing phosphate binder is taken, the patients should be in a neutral to slightly negative balance depending on stool and sweat output. In patients taking vitamin D calcitriol or its analogs, the intestinal absorption of calcium will be increased, and thus the maximum amount of calcium in the form of binders should probably be decreased. In patients with low turnover bone disease, the bone cannot take up calcium.

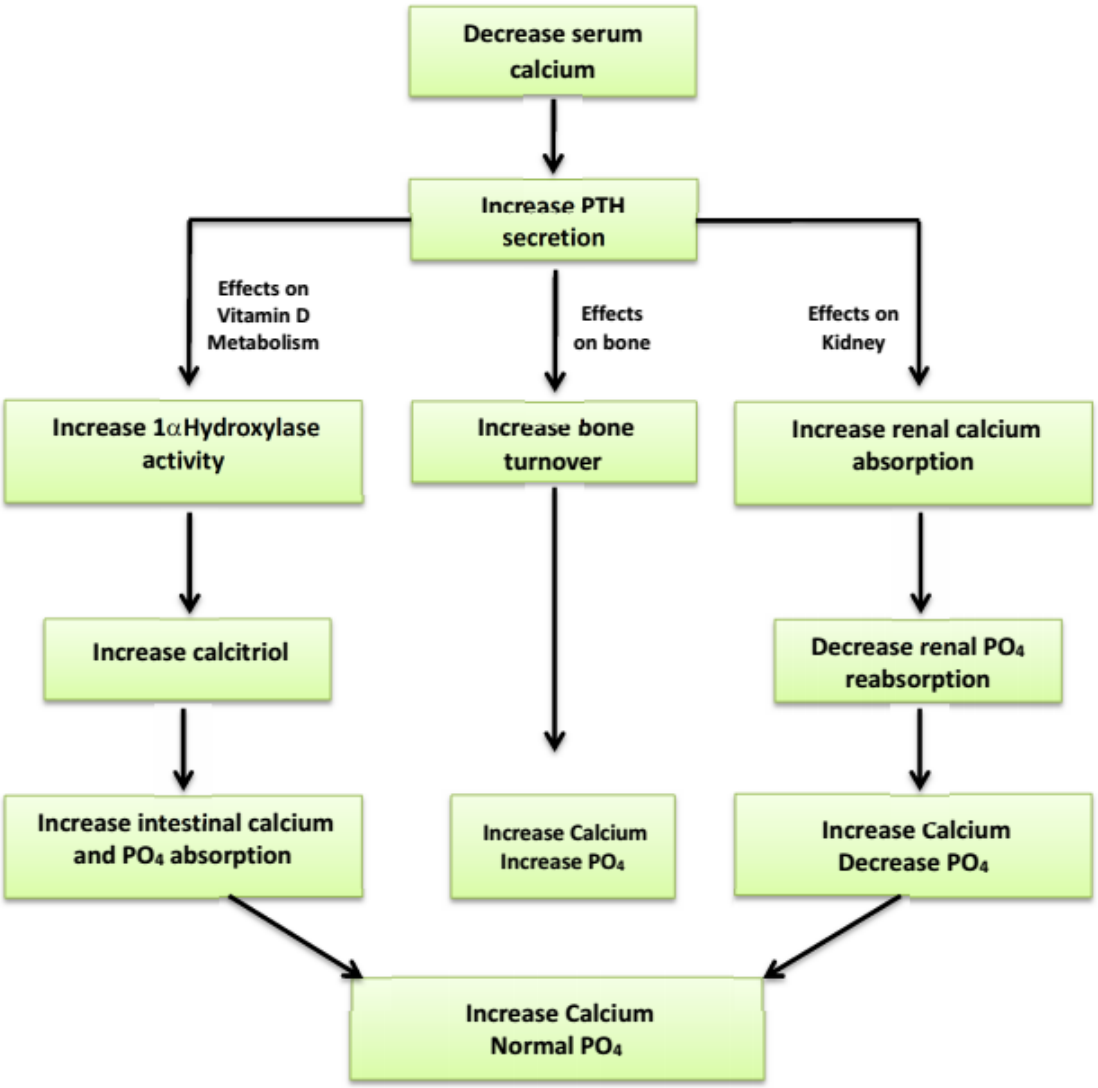

Fig 5. Normalisation of serum calcium by multiple action of PTH

Calcium Homeostasis

Calcium absorption across the intestinal epithelium occurs via a vitamin D–dependent transcellular and an independent paracellular pathway. In states of adequate dietary calcium, the paracellular mechanism succeeds, but the vitamin D–dependent pathways are critical in calcium deficient states. The transcellular absorption occurs via three steps: (1) the entry of calcium from the lumen into the

cells via transient receptor potential vanilloid (TRPV) channels, of which TRPV6 is most important; (2) the intracellular calcium then associates with calbindin9K to be "carried" to the basolateral membrane; and calcium is removed from the enterocytes predominately via the calcium–ATPase, with the Na/Ca exchanger playing a minor role.

The duodenum is the major site of calcium absorption, although the other segments of the small intestine and the colon also contribute to net calcium absorption. All of the key regulatory components of active calcium transport, TRPV, calbindin, Ca-ATPase, and Na/Ca exchanger are regulated by calcitriol. In the kidney, 60%-70% of calcium is reabsorbed passively in the proximal tubule driven by a trans epithelial electrochemical gradient that is generated by sodium and water reabsorption. In the thick ascending limb, another 10% of calcium is reabsorbed via paracellular transport.

Calcium Sensing Receptor activation in this segment inhibits calcium absorption. This paracellular reabsorption also requires the specific protein paracellin-1, and genetic defects in paracellin-1 lead to a syndrome of hypercalciuria and hypomagnesaemia.

However, the more regulated aspect of calcium reabsorption occurs via trans cellular pathways that occur in the distal convoluted tubule and connecting tubule. Calcitriol regulates all of these transport proteins. The epithelial uptake of calcium occurs via TRPV5 and TRPV6 transporters located on the apical membrane of the intestine and distal nephron segments, which are highly selective for calcium.

There are multiple regulators of TRPV5: acid pH decreases TRPV5 activity by altering membrane trafficking; intracellular calcium acts as a negative feedback switch; PTH and 1,25(OH)2D affect gene transcription; tissue kallikrein increases TRPV5 plasma membrane expression; Klotho modifies TRPV5 channel glycosylation, which in turn induces membrane accumulation; and activation of the CaSR stimulates TRPV5 activity. The multiple regulatory pathways involved support a critical role of TRPV5 in kidney distal segment calcium reabsorption.

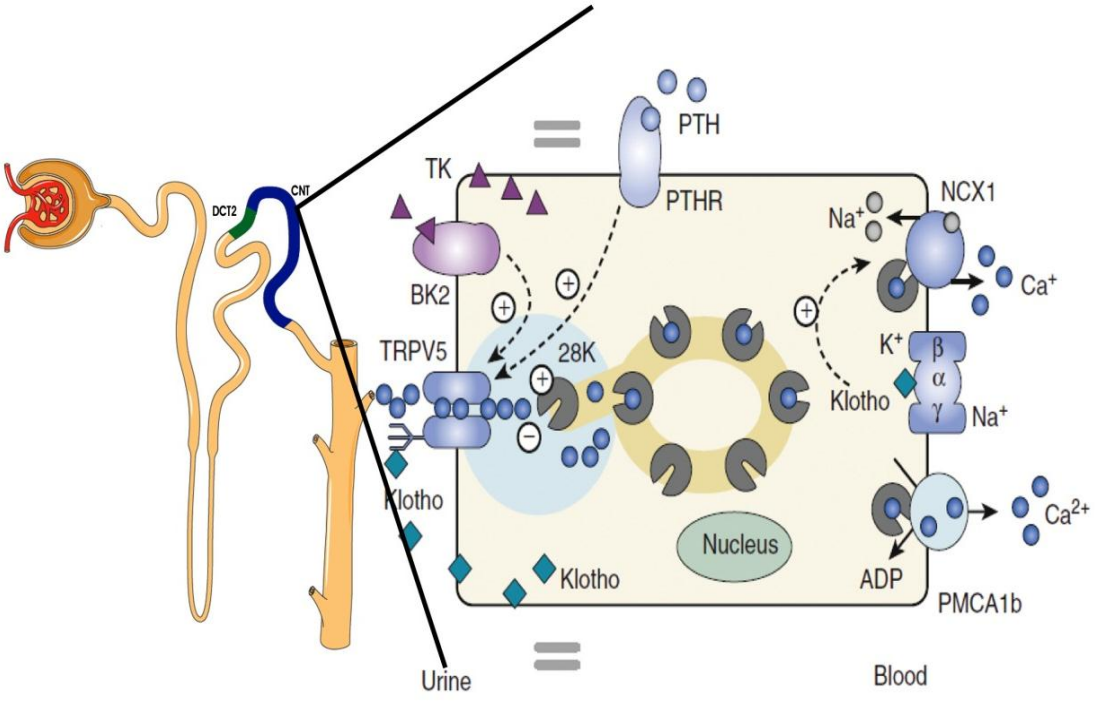

Fig. 6 Calcium Active Transport

This picture explains about epithelial calcium active transport. Calcium active transport occurs mainly in DCT [distal convoluted tubule] and CNT [connecting tubule].TRPV5 is an epithelial calcium channel is present mainly in

DCT and CNT. TRPV5 co-localizes with calbindin[28K], NCX1[sodium calcium exchanger] and PMCA1b[plasma membrane ATPase]. Calcium enter via apical TRPV5 and then it is buffered by 28K. And it reaches the basolateral membrane, where it is released and extruded by NCX1 and PMCA1b. Basolateral membrane also have parathyroid hormone receptor [PTHR] and sodium potassium ATPase consisting of alpha, beta and gamma subunits. PTH will activate PTHR which in turn activate TRPV5 and the entered calcium controls the level calcium transporters. TK [urinary tissue kallikerin] will cause activation of BK2 [bardykinin receptor 2] which also activates TRPV5. Klotho directly activate TRPV5. Entered calcium has a negative feedback.

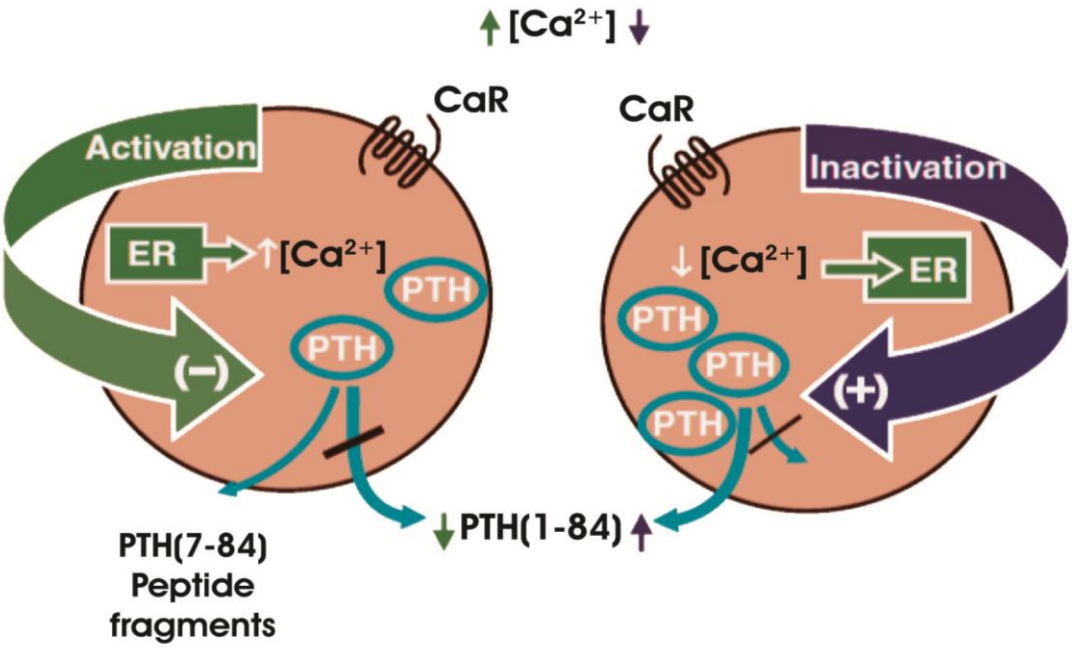

Fig-7- Calcium Sensing Receptor

CaSR[Calcium sensing receptor]. Increased serum calcium level will activate the CaR, which activate intracellular signaling and help in mobilization of intracellular calcium from endoplasmic reticulum[ER] and thereby inhibits the PTH

synthesis. Similarly decrease in serum calcium level will inhibit the intracellular signaling and leading to increase in PTH synthesis.

FIBROBLAST GROWTH FACTOR-23:

FGF23 levels are raised in patients with X-linked hypophosphatemia.[15,17] The features include defective calcification of cartilage and bone, leading to rickets, osteomalacia and growth retardation. This is the skeletal phenotype. The renal phenotype includes impaired renal tubular reabsorption of phosphate and abnormal regulation of 1,25(OH)2D production, which in turn leads to hypophosphatemia. The hypophosphatemia seen here is resistant to phosphorous and vitamin D therapy.[18]

FGF23 is also a phosphaturic factor. Seen mainly in patients with tumor-induced osteomalacia (TIO) .[19-22] Oncogenic osteomalacia /TIO is an acquired paraneoplastic disease. Here hypophosphatemia is caused by renal phosphate wasting .[21]Reductions in circulating FGF23 concentrations occurs with the removal of tumor and also with the correction of the hypophosphatemia.[15,16]

Missense mutations in the FGF23 gene as the causative factor of autosomal dominant hypophosphatemic rickets (ADHR). It involves short stature, bone pain, fracture and lower extremity deformity.[22,23] It is caused by gain-of-function mutations (R176Q, R179Q and R179W) leading to FGF23 resistance to proteolytic cleavage. Hence levels of circulating FGF23 concentrations are increased which leads to phosphate loss.[19,20,25]

Inactivating mutations in the FGF23 gene leads to degradation of FGF23 into inactive C-terminal fragments and causes familial tumoral calcinocis (FTC). FTC

includes hyperphosphatemia, elevated 1,25(OH)2D levels and soft tissue, vascular calcifications.[26-28] All these show that FGF23 acts as a central hormonal regulator of phosphate homeostasis.

FGF23:

Fibroblast growth factor (FGF) is a group of growth factor which have a common core region with 120 amino acids where the N- and C-terminal residues differ. Seven subfamilies of human FGFs[29-33] have been identified.FGF23 is the 23rd discovered FGF[34] and hence the name. The gene for FGF 23 is located on chromosome 12p13. FGF23 is produced as a 32kDa protein. It is made up of 251 amino acids. The 24 amino acid hydrophobic terminus acts as a signal sequence (Figure 2).[33, 34]

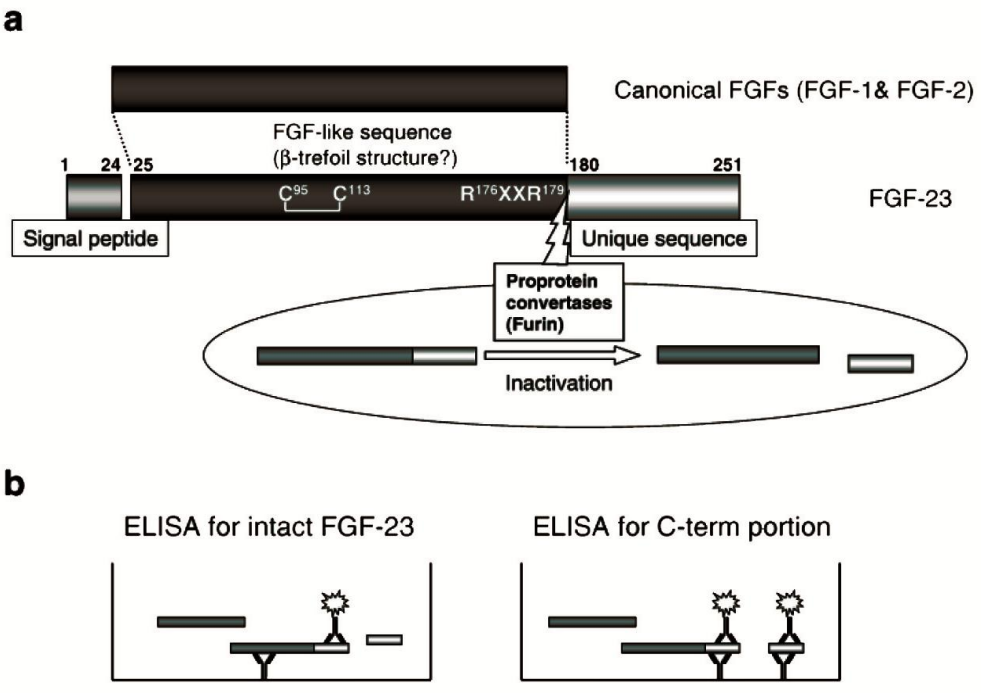

Fig-8 Structure of FGF-23

O-linked glycosylation of the threonine residue at position 178[19] is required for the transportation from the Golgi apparatus and the post-translational modification of FGF23 is carried out by the enzyme GALNT3 [35] the inactivating mutations of which causes FTC and hyperphosphatemia.

The T ½ of FGF23 is 58 minutes.[36] The biologically active protein is cleaved by a furin-like enzyme at its 176RXX179R motif, which results in generation of biologically inactive N- and C-terminal fragments.[37,, 38] FGF23 has structural similarity to the members of the FGF19 subfamily like FGF19 and FGF21 .[33] The FGF19 subfamily members have low affinity for heparin, in contrast, members of other FGF subfamilies bind to heparin sulfate present on the cell surface of producing cells. Assays for measurement of human FGF23 are available.

FGF23 levels (full length)are estimated by sandwich ELISA technique where two kinds of monoclonal antibodies detect the simultaneous presence of both the N-terminal and C-terminal portions of FGF23.[39,40] The C-terminal assay recognizes full-length as well as processed C-terminal fragments of FGF23.

Structure of FGF-23

There are seven subdivision of human FGFs.[42] There are 3 proteins in FGF-19 subfamily - FGF-19, FGF-21, and FGF-23 they all have different physiological functions. Phosphate homeostasis and calcitriol blood levels are regulated by FGF-23; The expression of enzyme cholesterol 7-a-hydroxylase (CYP7A1) is the first rate limiting enzyme in bile acid synthesis and which is inhibited by FGF-19.[43] FGF-21 helps in increasing insulin-independent glucose uptake in adipocytes and at the same time it will reduces triglyceride levels. [44]

A disulfide bond is present in FGF-19, FGF-21, and FGF-23 which is not available in other subgroups. This bond help FGF-23 distribution in the bloodstream and also to guide its functions.

Bone cells, mainly osteoblast secretes FGF-23 which is a 251-amino acid protein (26 kDa).[44] FGF -23 is composed of an amino-terminal signal peptide (residues 1–24) and it is followed by a carboxyl-terminal extended sequence (residues 181–251) and "FGF-like sequence" (residues 25–180) and which is specific compared with other sub groups of the FGF family[45] The half-life of an intact FGF-23 is 58 min[46] in healthy individuals. FGF-23 acquire its biological effects through activation of FGF receptors (FGF-Rs) and it is Klotho dependent. FGF-23 binds with a Klotho/FGF-R complex with greater affinity than does FGF-R or Klotho separately.[47] Klotho is a 130-kDa trans membrane b-glucuronidase and it has the ability of hydrolyzing steroid b-glucoronides.

The name was given to honor the God Klotho, one of the Moirae (the fates) from Greek mythology and the mice which are deficient in Klotho manifest a syndrome which will be similar to severe atherosclerosis and accelerated human aging. Because FGF-23/mice show phenotypes similar to Klotho/mice, a single signaling pathway has been proposed.[48,49]

Renal tubule, parathyroid, and choroid plexus have expression Klotho gene. Interestingly, the renal expression of Klotho is largely limited to the distal tubules, which is also the site for initial FGF-23 binding and signaling.[50,51] However, renal phosphate reabsorption happens primarily in the proximal tubules, and it is an enigma how FGF-23 signaling in the distal tubule gets converted into decreased phosphate reabsorption in the proximal tubules.

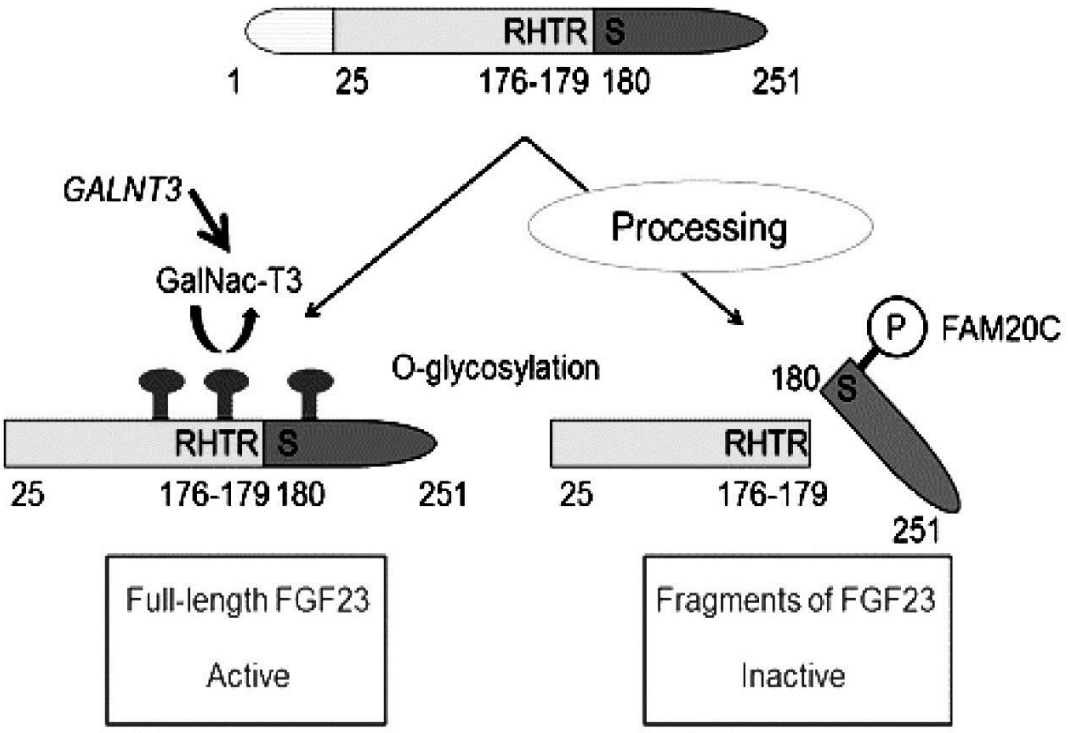

Fig-9 [52] Structure and post transitional modification of FGF-23 protein. It is protein with 251 amino acid

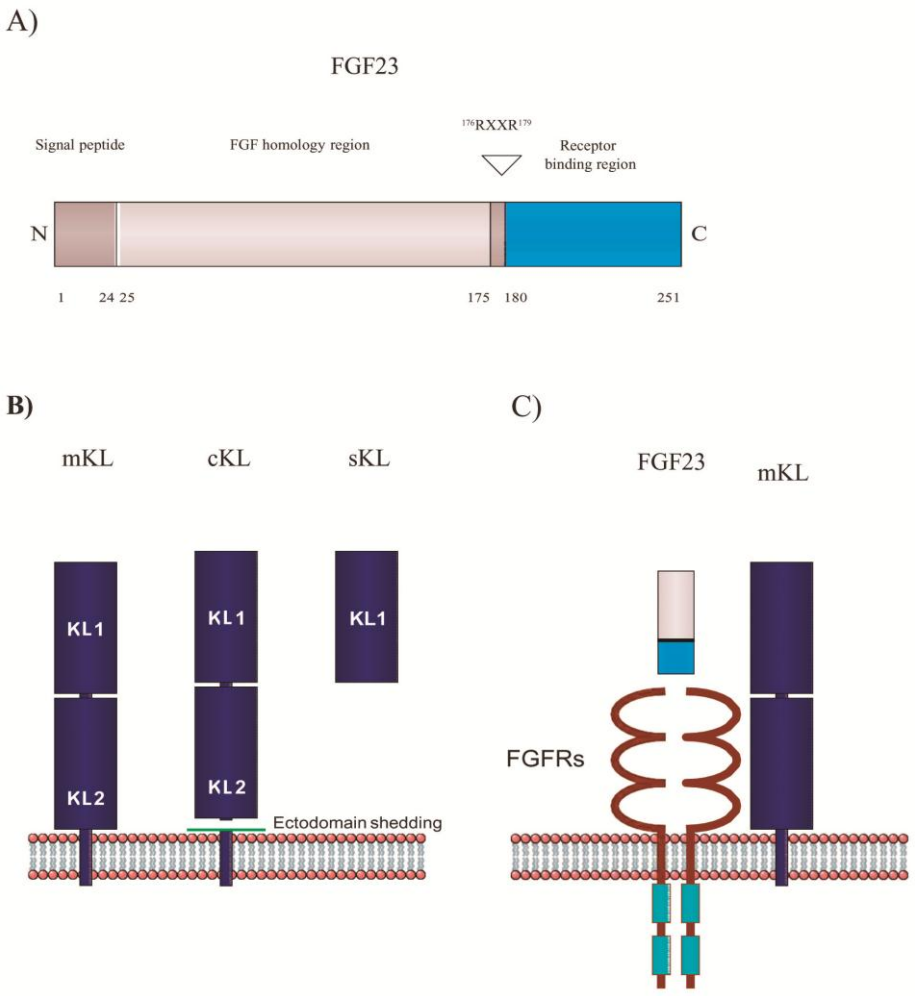

Fig-10[53]. Fibroblast growth factor-23 (FGF23), the three isoforms of Klotho and the FGF23–FGF receptor (FGFR) - Klotho complex.

A) FGF23 is 251 amino acid protein, which have N- terminal region, FGF homology region and the unique C-terminal receptor-binding region, where interaction with Klotho happens. It can divided into 2 fragments at position ^{176}RXXR^{179}.

B) Membrane-bound Klotho (mKL). Soluble Klotho[cKL] is acquired when mKL is cleaved by secretases at the cell surface Another form of soluble Klotho (sKL) is produced by alternative splicing at exon 3.

C) IFGF23 binds with receptor complex of Klotho and a FGFR which will activate downstream signaling. Dimer to activate downstream signaling

PHYSIOLOGY OF FGF23

The FGF19 subfamily members FGF19, FGF21, and FGF23, function as hormones that take action on particular target organs and controlled diverse metabolic processes.[54] FGF19 expression influence upon intestinal epithelial cells in and react to bile acid which is liberated into the intestinal lumen. FGF19 that acts upon hepatocytes and on the gall bladder as a crucial component in a postprandial negative feedback loop for bile acid synthesis and liberated.[55, 56] In contrast, FGF21 expression influenced upon fasting in the liver.[40, 41] FGF21 helps in glucose uptake in adipocytes[59] and reduced fat storage through its activity and which in turn stimulates lipolysis.[57, 58]

FGF23 which is released from bone (primarily in osteoblasts and osteocytes) [27, 38, 60] and is essential for mineral metabolism, where it has three different functions. First, FGF23 is a phosphaturic hormone.[61, 62] FGF23 generate phosphaturia in proximal tube of kidney through reduced expression and endocytosis of the sodium-phosphate co-transporters NPT2a and NPT2c .This is capable by activation of the mitogen-activated protein kinase (MAPK) pathway.[15] Second, as against parathormone-induced phosphaturia, FGF23-induced phosphaturia does not cause up regulation of 1,25(OH)2D production. In divergence, FGF23 suppresses renal 1-alpha- hydroxylase (1-OHase), as a result to reduced conversion of 25- hydroxyvitamin D (25(OH)D) to its active metabolite 1,25(OH)2D. FGF23 again decreases 25(OH)D and 1,25(OH)2D levels by stimulating 24- hydroxylase, which is needed for vitamin D degradation.[63] Third, in

the parathyroid, FGF23 decreases parathormone expression and secretion [64, 65], and rises 1-hyroxylase mRNA levels, which distinct with the negative effects of FGF23 on 1-OHase expression in the kidney.[65]

Regulation of FGF23

Although the regulation of FGF23 impression in bone remains disputable, there are several other factors that linked directly or indirectly to influence serum FGF23 level.

FGF23 function

FGF-23 has action on kidney and on parathyroid gland. FGF-23 acts on proximal tubule of the kidneys there by decreasing the reabsorption of phosphate by down regulation of Npt2a and Npt2c[66]. FGF-23 not only inhibits the vitamin D activating enzyme CYP27B1 but also activates 1, 25-dihydroxy Vit D leading to the accelerated breakdown of Vit D. this causes a reduction in the levels of circulating $1,25(OH)_2D$[67]. FGF-23 lowers the synthesis and secretion of PTH in the parathyroid gland and in contrast to the kidneys where it executes the expression of CYP27B1.[68,69]

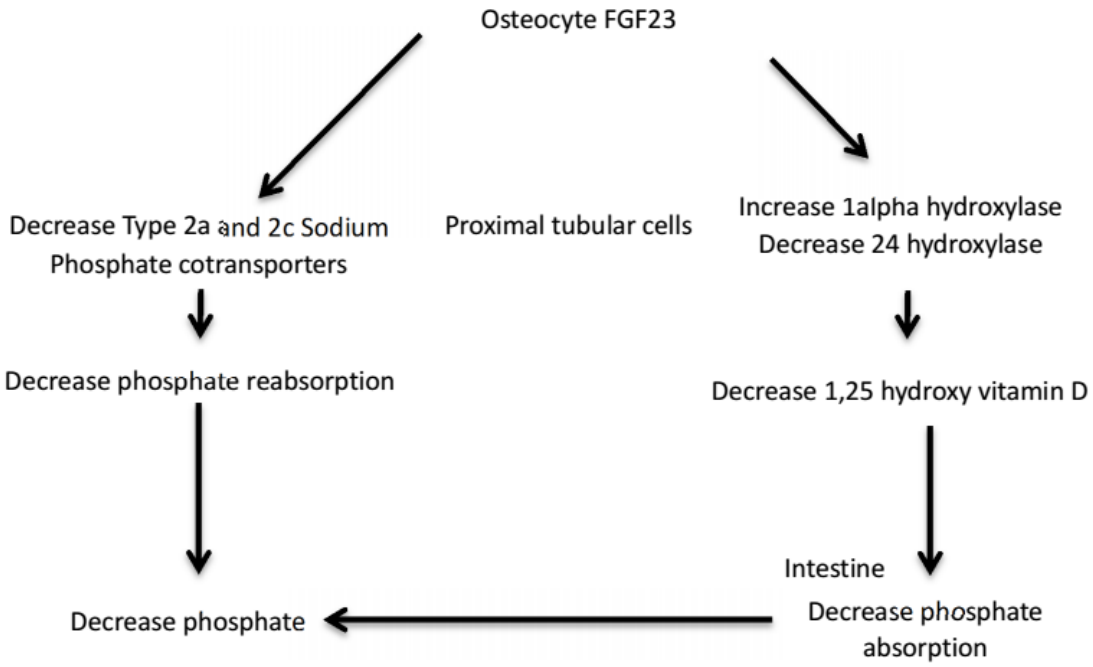

Fig-11 Function of FGF 23[70]

FACTORS AFFECTING FGF-23

PHOSPHATE

High-level of concentration of phosphate induce FGF23 promoter activity in vitro.[71] In vivo studies revealed a consonant rise in FGF23 levels in turn to phosphate loading.[72,73] In human, FGF23 levels are affected by alteration in dietary phosphorus[74-76] and rise in reaction to raised serum phosphate levels in patients with chronic hypoparathyroidism.[77] In healthy male participants, acute rise and decrease of serum phosphate level did not affect circulatory FGF23 levels, which demonstrate that FGF23 is not associated in the rapid adaptation of phosphate homeostasis.[78,79]

Also, while dietary phosphorous load affects FGF23 levels, FGF23 levels were unaffected by non-dietary intercession that increased serum FGF23 levels such as intravenous phosphorous infusion.[79] Thus, it is still unclear how phosphate in

sensed; the phosphate sensor is yet to be discovered and it is still not fully understood what triggers FGF23 actions.

Vitamin D:

The exposure of osteoblast cultures to 1,25(OH)2D and in vivo administration of calcitriol stimulates the production of FGF23 in bone and osteoblasts. This is not dependent on serum phosphate and PTH.[73, 80, 81] It is mediated by a vitamin D responsive element (VDRE) which is seen in the FGF23 promoter.[80]In contrast to this, VDR null mice have been found to have very low serum FGF23 and they are seen to not to respond to the 1,25(OH)2D administration.[73,82,83,84] Intravenous calcitriol therapy in humans increases serum concentrations of FGF23.[85]

Parathyroid hormone:

The levels of FGF 23are raised in primary hyperparathyroidism in an animal model, and was found to decrease after removal of parathyroid. The effect of PTH on circulating FGF23 in human is still unclear and results contradicting each other are seen.[86-90] In healthy male volunteers, an 24-hour intravenous infusion of PTH leads to a significant increase in FGF23,But, this effect may be due to the simultaneous increase in 1,25(OH)2D.

Genetic factors:

Genetic defects can both directly and indirectly result in the overstimulation of serum FGF23 levels. This is explained as the case in ADHR [91-92], ARHR [93-94] and XLH.[15-17,95,96] XLH is caused by inactivating mutations of PHEX which is a phosphate-regulating gene which is homologous to endopeptidases on the X

chromosome.[15, 97-99] The mouse cDNA sequence of PHEX is very much similar to that of humans. The deletion of the PHEX gene in the Hyp mouse hence results in an animal model of XLH. [100,101]

Increased FGF23 production by osteocytes in bone result from mutation in PHEX. This is seen by elevated FGF23 levels in XLH patients[15-17] and also in the Hyp mouse. Inhibition of FGF23 action in these mice is achieved by crossing the Hyp mice with the FGF23 null mice.[102] It can also be achieved by administration of anti-FGF23 neutralizing antibodies to Hyp mice.[103] This corrects the hypophosphatemia and the normal serum 1,25(OH)2D levels in these mice. This also decreases the rachitic bone phenotypes typically observed in Hyp mice, such as impaired longitudinal elongation and defective mineralization and abnormal cartilage development. Increase action of FGF23 is the reason for the hypophosphatemic rickets which observed in Hyp mice.

Fibroblast growth factor receptors and FGF23 signaling:

The identification of physiologically relevant FGF23-receptor has been difficult. A large number of in vitro studies have failed to reproduce the effects of FGF23 on mineral metabolism. But still binding studies have supported a low-affinity binding to FGFR1c, FGFR2c, FGFR3c, and FGFR4 mainly in the presence of sulfated heparins and the expression of certain glycosaminoglycan's (GAGs). [104]

Till the discovery of type I membrane-bound alpha-Klotho (Klotho), FGF23 signaling was not completely understood. Klotho binds to multiple FGFRs and forms a Klotho-FGFR complex. This complex binds to FGF23 with more affinity when compared to FGFR or Klotho alone. Klotho signaling also enhances the ability

of FGF23 to induce phosphorylation of FGF receptor substrate.[105] The target specificity of all the FGF19 subfamily members is estimated by the tissue distribution of Klotho.[106] In contrast to FGF23, FGF19 and FGF21 both need beta-Klotho for their action.

KLOTHO gene encodes a 130 kDa single-pass trans membrane protein which has a short cytoplasmic domain (10 amino acids). It is predominantly expressed in the kidney. The mice which carried a loss-of-function mutation in the KLOTHO gene developed a syndrome which closely resembles human aging, including reduced life span, skin and muscle atrophy, osteoporosis, arteriosclerosis, and pulmonary emphysema. [107] Contrary to that, overexpression of the KLOTHO gene increases the life span and also resistance to oxidative stress in mice. [108-111]

Klotho is an important FGF-receptor co-factor for FGF23. The evidence is produced by common phenotypes developed by Klotho-deficient and FGF23-deficient mice, including shortened life-span, growth retardation, infertility, muscle atrophy, hypoglycemia, and vascular calcification in the kidneys. They both have increased serum levels of phosphate.[112, 113] Injecting wild- type mice with an anti-Klotho monoclonal antibody induces FGF23 incompetence.[114]

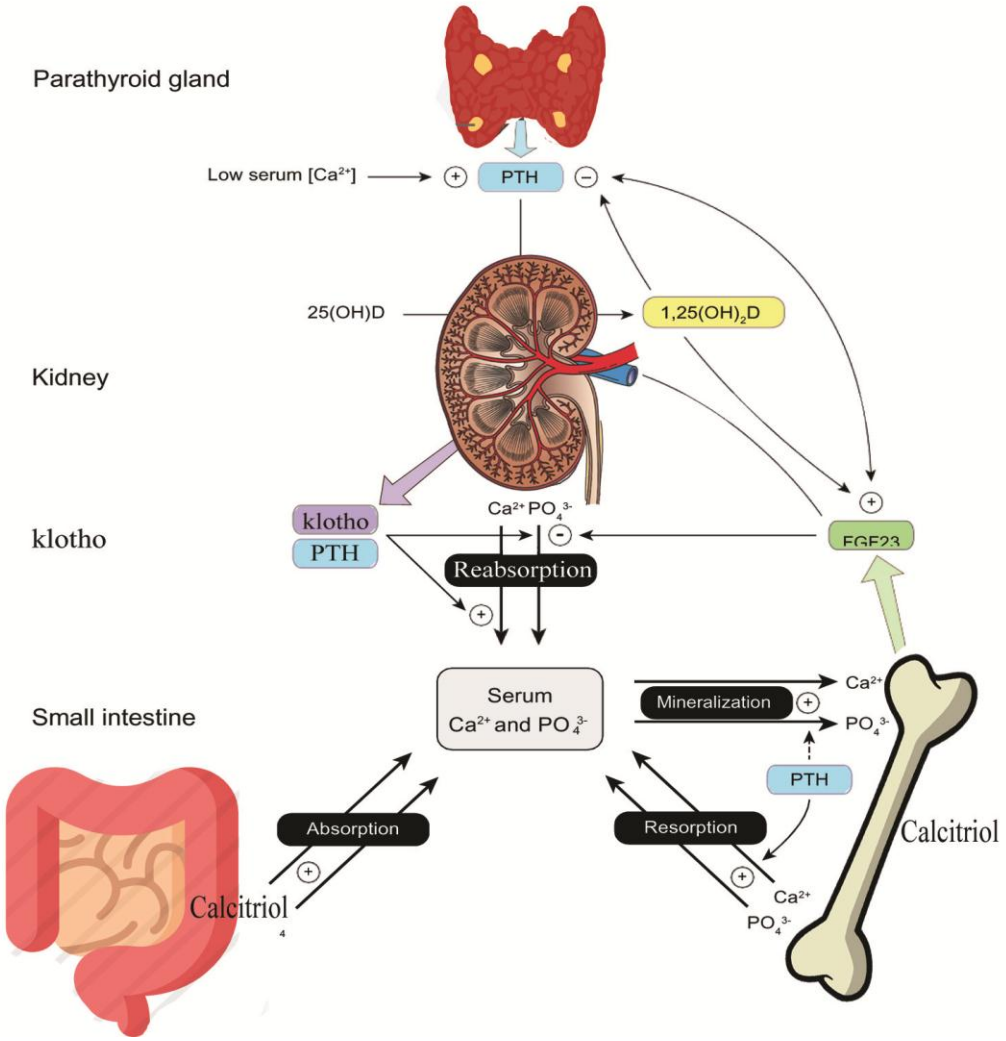

Fig-12.[115,116] calcium and phosphate metabolism and its endocrine regelation. Parathyroid gland, kidneys, bone and intestine play an important role in calcium and phosphate metabolism. Parathyroid hormone (PTH), FGF-23, soluble Klotho and vitamin D3 are important hormone regulators. They regulate each other through negative feedback. When serum calcium level is parathyroid gland releases PTH. PTH will increase calcium reabsorption in kidney directly and through the activation of vitamin D3 it will increase the intestinal calcium absorption and also it will increase the bone resorption. Klotho is phosphaturic and calciotropic hormone released from the kidneys. When there is high level phosphate in the serum it will induce the secretion of FGF23.

Bone Manifestations of CKD

The bone disorders can be classified into high bone turnover disorder and low bone turnover. The high bone turnover disorder have increased PTH levels whereas low bone turnover disorder have either normal or low PTH. The high-turnover bone disorder occurs due to following abnormal mineral metabolism:

(1) In CKD there will be decline in the level of GFR (less than 60ml/min) it will lead to decrease in the excretion of phosphate and thereby resulting in phosphate retention

(2) The phosphate retention will stimulate osteocytes and parathyroid gland. Their stimulation lead to the synthesis of FGF23 and PTH respectively; and

(3) FGF23 will suppress the production of calcitriol leading to decreased levels of calcium. Retained phosphate as well as kidney failure also lead to decreased calcium levels.[5.]

FGF-23 increase renal phosphate excretion. Recently studies have shown that levels of FGF-23 increased in the early stages of renal failure. By three ways FGF-23 may defend normal serum phosphorus:

(1) in kidneys it will increase phosphate excretion (2) stimulation the parathyroid gland to produce PTH, thereby increasing renal phosphate excretion and (3) inhibit the synthesis of vitamin D3 from the kidneys, leading to reduced level of phosphorus absorption from the GI tract. Significantly, high levels of FGF-23 is a risk factor for left ventricular hypertrophy and mortality in CKD, dialysis, and renal transplant patients.[5]

Stimulation of parathyroid gland will lead to hyperparathyroidism which increases bone turnover. High bone turnover in turn leads to osteitis fibrosa cystica.

Abnormal osteoid and bone marrow fibrosis are in bone histology. Brown tumor meant in last stages of the disease bone histology shows hemorrhagic elements along with bone cyst formation which will appear brown in color, hence the name. Brown tumors, bone pain and fragility, compression syndromes, and erythropoietin resistance in relation to the bone marrow fibrosis are the clinical manifestations of hyperparathyroidism. Fibrosis of cardiac muscle, weakness of the muscle and other nonspecific constitutional symptoms can occur due to the high levels of PTH.[5]

Low-turnover bone disease is classifies into two 1. Adynamic bone disease 2. Osteomalacia. Nowadays Adynamic bone diseases are more prevalent among diabetics and the elderly. In these diseases, there will be low bone mineralization and low bone volume. Inhibition of PTH can be due to high calcium exposure from the dialysis solution having high calcium content or calcium phosphate binders and excessive use of VIT-D. Bone fracture, bone pain, cardiac and vascular calcification are the complications of adynamic bone diseases. The term "Tumoral calcinosis'' meant calcium getting precipitated in soft tissues into large concretions.[5]

Calcium, Phosphorus, and the Cardiovascular System

The studies have shown that there is a strong association between increased cardiovascular mortality rate and hyperphosphatemia in patients with stage 5 CKD and even in patients with earlier stages of CKD. Hypercalcemia and hyperphosphatemia are associated with increased vascular calcification. Use of computed tomography (CT) and electron-beam CT scanning show that CKD patients have calcification of the media in coronary arteries and even heart valves than in patients without renal disease.[5]

Hyperphosphatemia and age are proportional to the magnitude of classification. Calcification is also associated with low bone turn over and low PTH levels. In patients with advanced kidney disease the calcium that is ingested cannot be deposited in bones with low turnover. Hence the calcium that is ingested get deposited at extra osseous sites like soft tissues, vascular beds.[141]

It is interesting in this regard that there is also an association between osteoporosis and vascular calcification in the general population. Hyperphosphatemia can cause a change in gene expression in vascular cells, leading to vascular calcification and even ossification.[5]

Other Complications of Abnormal Mineral Metabolism

Calciphylaxis (calcific uremic arteriolopathy) is one of the most catastrophic condition seen in patients with advanced stages of CKD. Livedo reticularis and patches of ischemic necrosis, especially over the legs, thighs, abdomen, and breasts proclaim to have calciphylaxis. Soft tissue and vascular calcification in association with vascular occlusion can be seen pathologically. Recently it is described that calciphylaxis is seen more frequently among patients with absence of severe hyperparathyroidism.[5]

Other causes have been suggested, including the increased use of oral calcium as a phosphate binder. Warfarin is commonly used in hemodialysis patients, and one of the effects of warfarin therapy is to decrease the vitamin K– dependent regeneration of matrix GLA protein. This latter protein is important in preventing vascular calcification.[5]

Warfarin is most commonly used in patients who are on hemodialysis.One of the functions of warfarin is to reduce the regeneration of matrix GLA protein which is vitamin K dependent. The GLA protein is vital in preventing the vascular calcification.[5]

Hence warfarin therapy is considered to be risk factor for calciphylaxis. If a patient has developed calciphylaxis then it is advice to stop warfarin. And we have to replace that patient with other form anticoagulants.[141]

TREATMENT FOR DISORDERS OF CALCIUM AND PHOSPHATE METABOLISM

Prevention is best way of management of secondary hyperparathyroidism and osteitis fibrosa. Because when parathyroid grows very large, it will be difficult to control the disease. We have to monitor the plasma concentration of phosphate in CKD patients. They be should be advised to be on low phosphate diet and phosphate binding agents. These are agents are taken with meals to reduce the absorption of phosphate.[5]

Calcium acetate and calcium carbonate are the examples of phosphate binders. Calcium-based phosphate binders can cause calcium accumulation and hypercalcemia, in patients with low-turnover bone disease. Non-calcium-containing polymers also function a phosphate binders they are Sevelamer and lanthanum. These drugs do not cause hypercalcemia in CKD patients and decrease the calcium deposition in the vascular bed.[5]

Calcitriol have suppressive effect on PTH secretion directly and also indirectly by increase the concentration of calcium. However, this may result in

hypercalcemia and/or hyperphosphatemia through increased GI absorption. Analogues of calcitriol are available (e.g., paricalcitol) which suppress PTH secretion without causing hypercalcemia.[5]

Recognition of the role of the extracellular calcium-sensing receptor has led to the development of calcimimetic agents that enhance the sensitivity of the parathyroid cell to the suppressive effect of calcium. This class of drug, which includes cinacalcet, produces a dose-dependent reduction in PTH and plasma calcium concentration in some patients. [5]

Current National Kidney Foundation Kidney Disease Outcomes Quality Initiative guidelines recommend a target PTH level between 150 and 300 pg/mL, recognizing that very low PTH levels are associated with adynamic bone disease and possible consequences of fracture and ectopic calcification.

CARDIOVASCULAR ABNORMALITIES

Cardiovascular disease is the leading cause of morbidity and mortality in patients at every stage of CKD. There is 10 – 200 fold increase of cardio vascular disease in patients with CKD depending on the stage of disease, age and sex. In stage 5 CKD patients there is about 30-45 percent risk of developing advanced cardiovascular compications.[5]

Ischemic Cardiovascular Disease

The presence of CKD is a major risk factor for ischemic cardiovascular disease and other occlusive disorders like stroke. Risk factors are classified into classic and CKD related. Classic risk factors include hypertension, hypervolemia, dyslipidemia, sympathetic over activity, and hyperhomocysteinemia. The CKD-

related risk factors comprise anemia, hyperphosphataemia, hyperparathyroidism, increased FGF-23, sleep apnea, and generalized inflammation. The inflammation cause increase in CRP (C-reactive protein) and cytokines and other acute phase reactants but it will decrease albumin and fetuin.

Low levels of fetuin may cause rapid vascular calcification, especially in the face of hyperphosphataemia. The inflammation can itself precipitate the damage to the kidneys. CKD may augment myocardial ischemia, including left ventricular hypertrophy and other micro vascular disease. During hemodialysis hypotension and hypovolemia also increase the cardio vascular risk. Patient instead of myocardial infraction can presents with congestive heart failure and all of its manifestations and sudden death.

Cardiac troponin levels are elevated in CKD patients without evidence of acute ischemia. So diagnosis of myocardial infraction became difficult. Serial measurement of cardiac troponin should preferred in patients with CKD. CPK-MB levels can measured in CKD patients instead of cardiac troponins.[5]

Heart Failure

Heart failure can be secondary to myocardial ischemia, left ventricular hypertrophy, and frank cardiomyopathy, the salt and water retention and ECFV overload. Heart failure can be diastolic or systolic or can be both. It can also lead to acute pulmonary edema. This process has been ascribed to increased permeability of alveolar capillary membranes as a manifestation of the uremic state, and it responds to dialysis. Other CKD-related risk factors, including anemia and sleep apnea, may contribute to the risk of heart failure.

Hypertension and Left Ventricular Hypertrophy

Hypertension is one of the most common complications of CKD. Left ventricular hypertrophy and dilated cardiomyopathy are among the major risk factors for cardiovascular morbidity and mortality in patients with CKD. Absence of hypertension signifies poor ventricular function and have poor prognosis. Anemia and AV fistula in hemodialysis patient can cause high output failure. Classic risk factors, such as hypertension, hyperlipidemia, and obesity, appear to have a better prognosis. Late-stage CKD, low blood pressure, reduced body mass index, and hypolipidemia indicate the presence of an advanced malnutrition-inflammation state, with poor prognosis. Exogenous erythropoiesis-stimulating agents can increase blood pressure and increase the need of antihypertensive drugs. Chronic ECFV overload will also lead to left ventricular hypertrophy.[5]

TREATMENT CARDIOVASCULAR ABNORMALITIES

Management of Hypertension

The aim of treating hypertension in CKD patients is to prevent the complications like cardiovascular disease and stroke. In CKD patients with diabetes or proteinuria >1 g per 24 h, blood pressure should be reduced to 130/80 mmHg. Salt restriction should be the first line of therapy. If not sufficient we can start on antihypertensive drugs. We can start with ACE inhibitors and ARBS because they slow the progression of kidney disease. These drugs can rarely cause AKI and they also have adverse effect of causing hyperkalaemia. Along with use of a kaliuretic diuretic [metolazone] can improve potassium excretion in addition to the benefit of lowering the blood pressure. Potassium-sparing diuretics should be used with high caution.

MANAGEMENT OF CARDIOVASCULAR DISEASE

There are several ways are there to treat the classic and CKD related risk factors. Theyare found to be effective in normal population but effectiveness is uncertain in patients with CKD especially those who are on dialysis. Hypertension, elevated serum levels of homocysteine, and dyslipidemia increase the incidence of atherosclerotic disease and they are treatable complications of CKD. Renal disease complicated by nephrotic syndrome is associated with atherogenic lipid profile and hypercoagulability, which will increases the risk of occlusive vascular disease.

The role of inflammation more important in patients with kidney disease, and the treatment of more traditional risk factors may result in only modest success. Modulation of classic risk factors may be the only way in treating these patients. They include Lifestyle changes, such as regular exercise, should be advised. Hyperlipidemia in patients with CKD should be managed according to national guidelines. If dietary measures are not sufficient, lipid-lowering medications can be preferred, such as statins, should be used. But thebenefits in CKD patients seems to be uncertain.

Fukagawa M et al[115] conducted a study on role of FGF 23 in CKD and in healthy individuals in 2005 and concluded that in CKD, FGF23 plays a crucial role in the pathogenesis of secondary hyperparathyroidism. Effects of FGF23 on other organs including bone and intestine are not clear yet.ss

Tobias Larsson et al[116] (2003) FGF-23 serum levels were highly elevated in CKD with a strong correlation between serum creatinine and FGF-23 concentration. Correlations were also seen between FGF-23 and phosphate, Ca2+, parathyroid

hormone (PARATHORMONE), and 1,25(OH)2D3. No changes in serum FGF-23 levels were observed in volunteers following ingestion of oral phosphate binders/low dietary phosphate intake, which led to a reduction in phosphate excretion or during the subsequent repletion with inorganic phosphate through oral phosphate and a normal diet. Circulating F GF-23 was highly elevated in patients with CKD and its concentration correlated with renal creatinine clearance. In healthy volunteers, FGF-23 levels did not change after phosphate deprivation or phosphate loading.

Danilo Fliser et al[117] (2007) It has not been firmly established whether disturbed Ca2+-phosphate metabolism affects development of CKD (CKD) in humans. In this cohort study of 227 nondiabetic patients with CKD, we assessed fibroblast growth factor 23 (FGF23) plasma concentrations in addition to other variables involved in Ca2+-phosphate metabolism, and we followed 177 of the patients prospectively for a median of 53 months to assess progression of renal disease. In the baseline cohort, we found a significant inverse correlation between glomerular filtration rate and both c-terminal and intact FGF23 levels . The 65 patients who experienced a doubling of serum creatinine and/or terminal renal failure were significantly older, had a significantly lower glomerular filtration rate at baseline, and significantly higher levels of intactparathormone, c-terminal and intact FGF23, and serum phosphate (all $P < 0.001$). Cox regression analysis showed that both c-terminal and intact FGF23 independently predict development of CKD after adjustment for age, gender, GFR, proteinuria, and serum levels of Ca2+, phosphate, and parathyroid hormone. The mean follow-up time to a development end point was 46.9 (95% CI 40.2 to 53.6) months versus 72.5 (95% CI 67.7 to 77.3) months for patients with c-terminal FGF23 levels above or below the optimal cut-off level of 104 rU/mL (derived by receiver operator curve analysis),

respectively. In conclusion, FGF23 is a good independent predictor of development of renal disease in patients with nondiabetic CKD. Its pathophysiological importance remains to be elucidated.[12]

Alper Kirkpantur et al[118] (2010) Plasma FGF-23 concentration is independently associated with LVMI and MPI in maintenance haemodialysis patients. Further prospective studies are neccesary to clarify if increased serum FGF-23 level is a marker or a potential mechanism for left ventricular involvement in patients with end-stage renal disease.[16]

Sherri-Ann M Burnett, Samantha C Gunawardene, et al[119]in 2006 conducted a study on regulation of FGF-23 by dietary phosphate in 66 men and women using two assays. Dietary phosphate restriction reduced FGF-23 and while loading phosphate caused increased FGF-23 significantly. They concluded that Dietary phosphate is a key regulator of circulating FGF-23 levels in humans.

Tarek Zakaria Osama A., et al, [120] in 2014 conducted a study to assess the effects of improving abnormal surrogate markers of metabolic bone disease on clinical outcomes in CKD patients. 40 adult ESRD patients on regular haemodialyis were included in this study. Each patient underwent full clinical evaluation, kt/v, hemoglobin level, serum creatinine, blood urea and lead electrocardiography. They concluded that Serum Calcium, serum phosphorus and iPTH are strong predictors of cardiovascular and all-cause deaths in HD patients. Improvement of biochemical parameters could not stop aortic abdominal calcification and increasing in carotid intima media thickness, but highly attenuate the development of calcification.

Bryan Kestenbaum, Joshua N. Sampson, et al,[121] in 2005, conducted a study on the relationship between serum phosphate and deaths in patients with CKD (CKD). A retrospective cohort study was conducted from 8 Veterans Affairs' Medical Centres located in the Pacific Northwest. CKD was defined by 2 continuously unusual outpatient serum creatinine measurements at least 6 months apart between 1999 and 2002. Patients who got chronic dialysis, those with a current or previous renal transplant, and those without a recent phosphate measurement were excluded. He analysed mortality risk increased linearly with each subsequent 0.5-mg/dl increase in serum phosphate levels. High serum phosphate levels were independently associated with increased deaths risk among that population of patients with CKD.

Orlando Gutierrez, Tamara Isakova, et al., in 2005 [122], did a study on Fibroblast Growth Factor-23 attenuates Hyperphosphatemia but Accentuates Calcitriol Deficiency in CKD. Several hypothesis were tested: that FGF-23 increases as renal function reduces and is linearly associated with serum phosphate levels; is correlated with raised phosphaturia independent of parathyroid hormone (parathormone); and is correlated with reduced calcitriol levels independent of renal function, hyperphosphatemia, and vitamin D stores. FGF-23,parathormone, 25(OH)D3, calcitriol, Ca2+, phosphate, and urinary fractional excretion of phosphate were measured in 80 CKD patients. They concluded that FGF-23 levels increase early in CKD before the development of serum mineral abnormalities and are independently correlated with serum phosphate and calcitriol deficiency. Raised FGF-23 can contribute to maintaining normal serum phosphate levels in the face of

advancing CKD but can worsen calcitriol deficiency and thus can be a central factor in the early pathogenesis of SHPT.

Geoffrey A. Block, Preston S. Klassen, et al.,[123] in 2004, did a study on mineral metabolism, mortality, and morbidity in maintenance haemodialysis. For determining associations among disorders of mineral metabolism, deaths, and morbidity in haemodialyis patients, data on 40,538 haemodialyis patients with at least one determination of serum phosphorus and Ca2+ during the last 3 months of 1997 were analyzed. Moderate to severe hyperparathyroidism (parathormone concentrations 600 pg/ml) was correlated with an increase in the relative risk of death, whereas more modest rise in parathormone were not. When examined collectively, the population attributable risk percentage for disorders of mineral metabolism was 17.5%, owing mainly to the high prevalence of hyperphosphatemia. Hyperphosphatemia and hyperparathyroidism were highly correlated with all-cause, cardiovascular, and fracture related hospitalization. Disorders of mineral metabolism are independently correlated with mortality and morbidity correlated with cardiovascular disease and fracture in haemodialyis patients.

Oniucci, Takeyoshi Yamashita, et al.,[124] did a study in 2006 to find if serum FGF-23 concentration is regulated by dietary phosphorus and thereby mediates the physiological response of serum 1,25(OH)2D to changes in dietary phosphorus. They studied 13 healthy men as inpatients during a 4-wk dietary phosphorus intervention study. They concluded that in healthy men, changes in dietary phosphorus within the physiological range of intakes regulate serum FGF-23 concentrations and suggested that dietary phosphorus regulation of 1,25(OH)2D production is mediated, atleast in part, by changes in circulating FGF-23.

Rodrigo B. Oliveira, Ana L.E. Cancela et al[125]n 2010 did a short-term 6-wk dose titration study evaluated the effect of two phosphate binders onparathormone and FGF23 levels in patients with CKD stages 3 to 4. Significant changes were observed for FGF23 only in sevelamer-treated patients. The study afirms the positive effects of early prescription of phosphate binders onparathormone control. Prospective and long-term studies are necessary to confirm the effects of sevelamer on serum FGF23 and the benefits of this decrease on outcomes.

Serge L. Ferrari, Jean-Philippe Bonjour et al.[126], in 2005 did a study on phosphate and Renal Phosphate Handling in Healthy Young Men and hypothesized that phosphate intake could impact FGF-23 concomitantly to the changes in renal Pi handling. FGF-23 was inversely related to renal Pi transport and serum calcitriol levels in healthy young men. The data suggest that FGF-23 can be implicated in the physiological regulation of Pi homeostasis in response to dietary phosphate changes, independent ofparathormone.

Nobuaki Ito,Seiji Fukumoto, et al. [127]in 2006 designed a study to find if acute changes of serum phosphate modulate FGF23 levels in human. Four healthy volunteers participated in the study. In the phosphate infusion study, dibasic potassium phosphate was infused at a rate of 10 mEq/h for 4 h, and serum FGF23 levels were measured for up to 6 h after the start of the infusion. In the carbohydrate study, partially hydrolyzed starch corresponding to 150 g glucose was ingested and FGF23 levels were measured similarly for Phosphate infusion highly raised and carbohydrate ingestion reduced serum phosphate levels, respectively. However,

FGF23 did not change by these manoeuvres. It is concluded that acute changes of serum phosphate do not modify FGF23 levels in the healthy human.

Thomas J Weber,1,2 Shiguang Liu,et al.,[128] did a study on 2003,to investigate if the circulating levels of the phosphaturic factor FGF23 are elevated in subjects with XLH. Fasting serum FGF23 levels and serum biochemical parameters were measured using a human FGF23 (C-terminal) ELISA assay in 11 subjects FGF23 levels were variably elevated in subjects with hypophosphatemia of unknown cause, one of which had tumor-induced osteomalacia (TIO). Excision of the tumor resulted in rapid reduction in serum FGF23 levels. These findings suggest that FGF23 has a possible role in mediating hypophosphatemia in XLH and TIO, but the varying levels of FGF23 in hypophosphatemic disorders and normal subjects indicates that serum phosphorus and FGF23 can also be independently regulated.

Per-Anton Westerberg, Torbjo, et al.[129], did a study on 2007 the regulation of FGF23 in CKD subjects with various degree of renal function. They analysed the relationship between FGF23, Pi, Ca2+, parathyriod hormone (PARATHORMONE), 25(OH) vitamin D3(25(OH)D3), 1,25(OH)2 vitamin D3(1,25(OH)2D3) and estimated glomerular filtration rate (eGFR). They concluded that serum FGF23 rises in CKD 4–5, in parallel with the emerging hyperphosphataemia.

H. Aggarwal, Deepa Jain, et al.,[130] in 2013, conducted a study on Bone mineral density in patients with predialysis CKD. The study included 75 patients. Patients were divided into three groups depending upon GFR. Serum creatinine , albumin, Ca2+, phosphate(PO4), alkaline phosphatase, parathormone and VitaminD were measured at baseline .There was negative correlation between Z-score and parathormone, and positive correlation with Vitamin D. Reduced bone density was seen

early in the course of CKD as estimated from reduced BMD levels, raised prevalence of osteoporosis and raised fracture is get worsened with development of CKD.

Y. Imanishi, M. Inaba, et al.,[131] in 2003, did a study on FGF-23 in patients with end-stage renal disease on haemodialyis. They tested the hypothesis that plasma FGF-23levels may be raised in hyperphosphatemia in patients with end-stage renal disease (ESRD) on maintenance haemodialyis. They measured plasma FGF-23 levels in 158 male uremic patients on maintenance haemodialyis. Plasma FGF-23 level exhibited significant and positive correlations with inorganic phosphate, intact parathyroid hormone (PARATHORMONE), corrected Ca2+, and duration of haemodialyis.

Fliser D.[132]**,** in 2007, did a study on if fibroblast growth factor 23 (FGF23) predicts development of CKD. they assessed fibroblast growth factor 23 (FGF23) plasma concentrations in addition to other variables involved in $Ca(PO4)_2$ metabolism this analysis showed that both c-terminal and intact FGF23 independently predict development of CKD after adjustment for age, gender, GFR, proteinuria, and serum levels of Ca2+, phosphate, and parathyroid hormone.

H.J. Hsu, et al.[133]**,** in 2009, did a study is to find the association of FGF23 and LVH and the prognostic value of FGF23 in chronic haemodialyis patients. LVH was also associated with higher levels of FGF23.They said that serum FGF23 level is independently correlated with LVH in our haemodialyis patients.

Inaba M., et al[134]**,** in 2006, did a study on *role* of fibroblast growth factor-23 in peripheral vascular calcification in non-diabetic and diabetic haemodialyis patients and the findings show that the plasma FGF-23 level is an independent factor

negatively correlated with peripheral vascular calcification in the hand artery, but not in the aorta, in both male non-DM and DM haemodialyis patients and also concluded that plasma FGF-23 level can provide a reliable marker for Moenckeberg's medial calcification in male haemodialyis patients, independent of its regulatory effect on Phosphate metabolism.

S. Pande, et al., [135] in 2006, did a study on FGF-23 and sFRP-4 in CKD and post-renal transplantation. Serum FGF-23, FRP-4, phosphorus and parathyroid hormone were measured in patients at all stages of CKD.sFRP-4 levels did not change with creatinine clearance or hyperphosphatemia in CKD or end-stage renal disease patients, and no relation was seen between post-transplant sFRP-4 levels and hypophosphatemia. FGF-23 levels correlated inversely with plasma phosphorus. They found that In CKD, FGF-23 levels rose with decreasing creatinine clearance rates and increasing plasma phosphorus levels.

M. Yilmaz, et al.,[136] in 2010, did a study on FGF-23 and vascular dysfunction in patients with stage 3 and 4 CKD. To analalyze the relationship between circulating FGF-23 levels and the response of forearm blood flow to ischemia (flow-mediated vasodilatation, FMD) and nitroglycerin, they found that endogenous inhibitor of NO synthase mediate the vascular effects of FGF-23 in patients with CKD.

J.J.Kazama, et al.,[137] in 2005, conducted a study on pretreatment serum FGF-23 levels predict the efficacy of calcitriol therapy in dialysis patients. Dialysis patients with plasma intact parathyroid hormone (PARATHORMONE) levels greater than 300 pg/mL were included in the study. They showed that FGF-23 was

the best screening test for identifying patients with future refractory response to calcitriol therapy.

P.U.Toreres, et al.[138], in 2008, did a study to see if bone mass correlates with the serum fibroblast growth factor 23 in haemodialyis patients. Circulating fibroblast growth factor 23 (FGF23) rises renal phosphate excretion, decreases bone mineralization and is markedly raised in haemodialyis patients. The study suggests that the effects of FGF23 on bone mineralization are mainly due to hypophosphatemia and not a direct effect on bone.

S. Nakanishi, et al.[139] in 2005, did a study on whether serum fibroblast growth factor-23 levels predict the future refractory hyperparathyroidism in dialysis patients. The regression analysis showed that only serum levels of FGF-23 were highly related to the prognosis of parathyroid function. They found Serum FGF-23 level was the most useful factor in predicting future development of refractory secondary hyperparathyroidism in long-term dialysis patients with mild secondary hyperparathyroidism.

MATERIALS & METHODS

METHODOLOGY

Study Design : Cross sectional study

Inclusion Criteria:

- Patient with CKD of the age above 18 years irrespective of the gender who are willing to participate

Exclusion Criteria :

- Patient who are not willing
- Or unable to give informed consent

No of Groups Studied

One group with CKD patients attending department of Medicine and Nephrology, Sree Mookambika Institute of Medical Sciences, Kulasekharam during the decided study period.

Sampling Population:

All the CKD Patients coming to department of General Medicine and Nephrology.

Sample Size Calculation:

$$= \frac{(1.96)^2 PQ}{d^2}$$

P=% of raised FGF23 in CKD patient [68%][17]

Q=100-P d=20% of P

Sample size = 46.17 = 47[20]

Sampling Technique used: Convenience Sampling

Study Procedure:

The kit uses 2 affinity-purified goat antibodies that bind at the carboxy terminal portion of fibroblast growth factor 23 (FGF23). One antibody is coated on to microtitre wells and the other is biotinylated. Horseradish peroxide conjugated to avidin and 3.3', 5,5'-tetramethylbenzidine (TMB) substrate provide the coloured product, which is read in a microtitre plate spectrophotometer.

- Ca2+ ions react with 5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex. This complex reacts in the second step with EDTA. The change in absorbance is directly proportional to the Ca2+ concentration and is measured photometrically.
- The method is based on the reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate (without reduction). The addition of an accelerator gives rise to a more rapid rate of reaction.
- Sample collection and storage:
  - We will take 5 ml of blood.
    a. Fasting-overnight (12-14 hours)
    b. Serum gel tubes should be centrifuged within 2 hours of collection.
    c. Red top tubes should be centrifuged and aliquoted within 2 hours of collection.

RESULTS

RESULTS

AGE

Distribution according to age of participants

The distribution of age in the study population ranges from 20 to 70 years. The mean age of study participants was 49.80 years and a SD of 12.03 years.

18 % of patient were in the age group of 20-39 years. 56 % in the group 40-59 years and 26 % in 60-79 years.

Table 1 Distribution according to age of participants

Age group	Frequency	Percent
20-39	9	18.0
40-59	28	56.0
60-79	13	26.0
Total	50	100.0

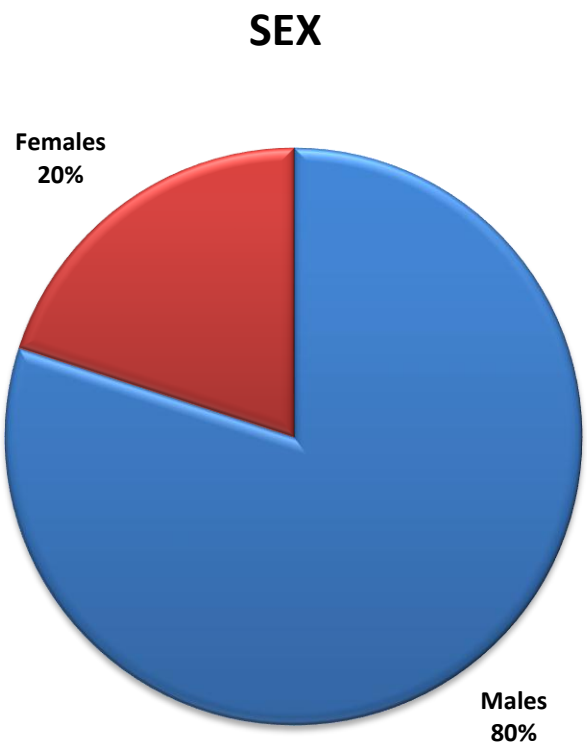

Fig-13 Distribution of gender in the study population

Pie Chart showing the distribution of gender in the study population.

80 % of the study population were male and only 20 % of the people were female

PERIPHERAL VASCULAR DISEASE

Bar chart showing the Distribution of Peripheral vascular disease among the study population

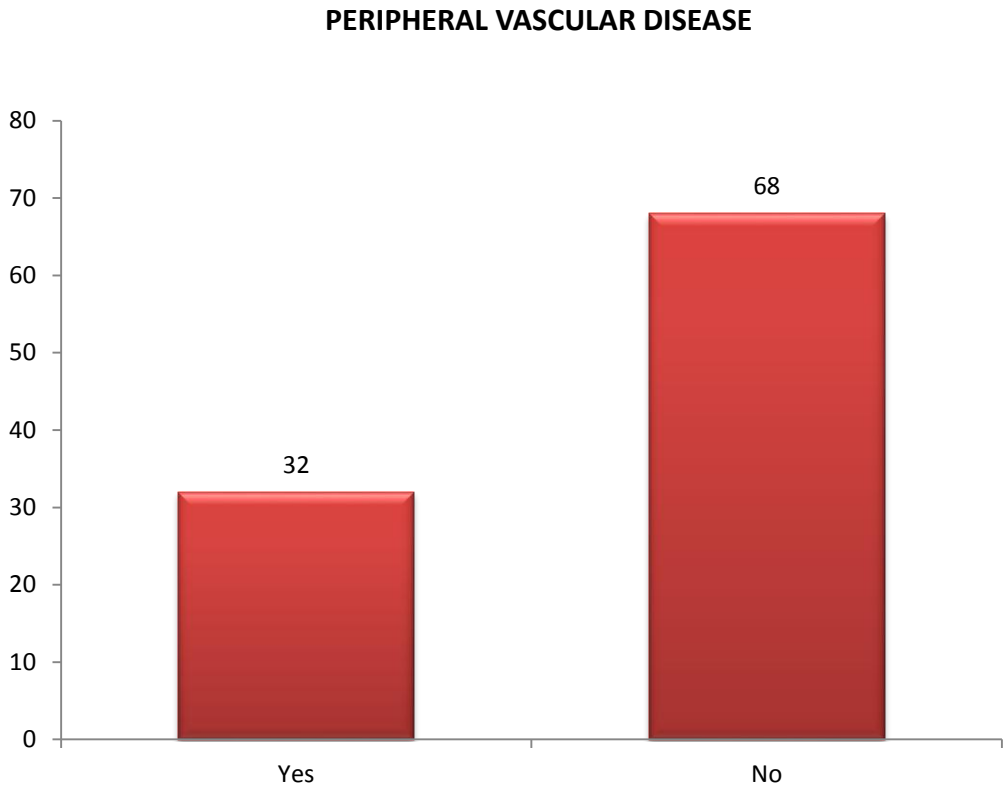

Fig-14 PERIPHERAL VASCULAR DISEASE

16(32%) of the study subjects had peripheral vascular diseases

CEREBROVASCULAR ACCIDENTS

Pie Chart showing the distribution of cerebrovascular accidents in the study population

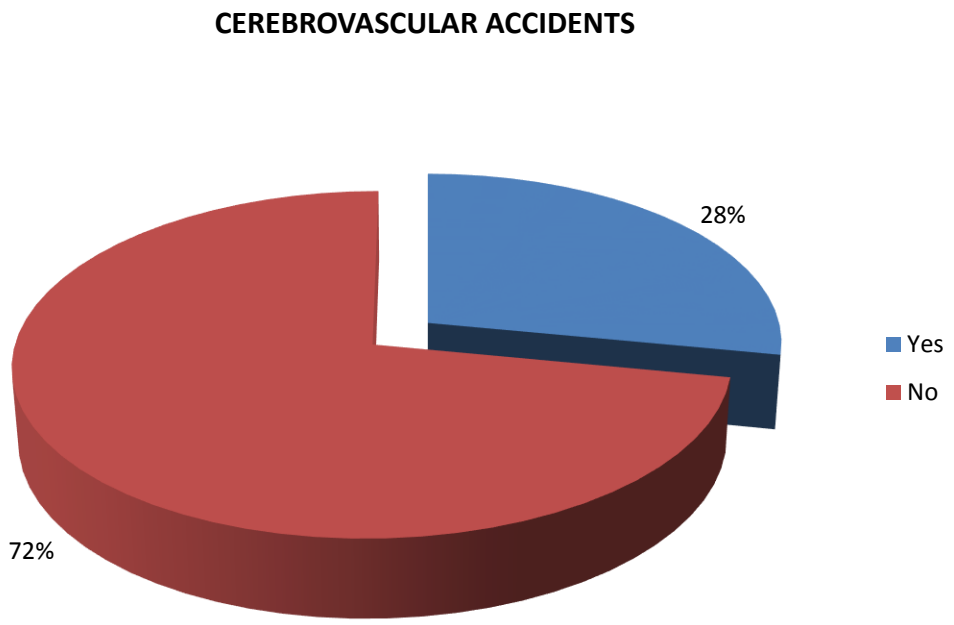

Fig -15 CEREBROVASCULAR ACCIDENTS

Out of the 50 study subjects 14(28%) of the study subjects had cerebrovascular accidents.

LEFT VENTRICULAR HYPERTROHPHY

Pie chart showing the distribution of LVH in the study population

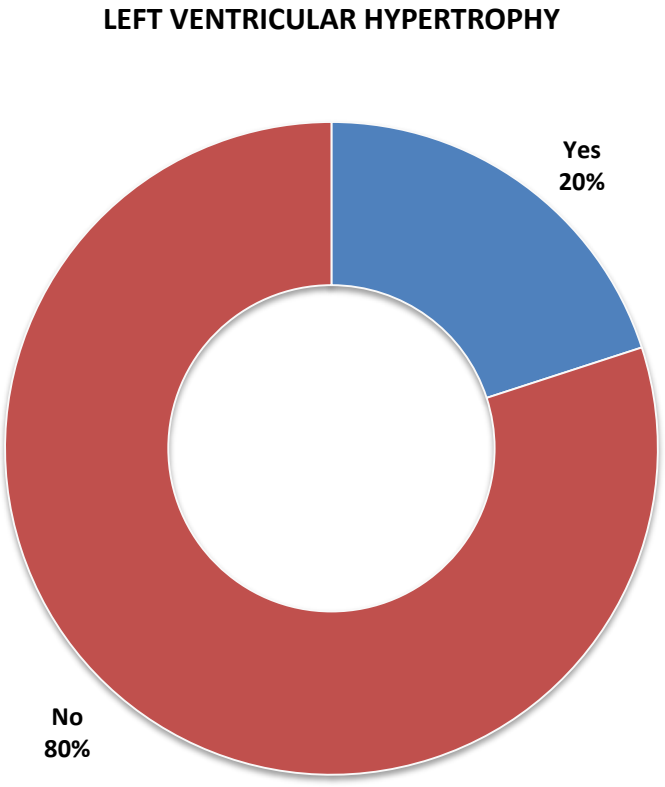

Fig.16 LEFT VENTRICULAR HYPERTROHPHY

Among the CKD patients 10 (20%) had left ventricular hypertrophy.

LV DYSFUNCTION

Pie chart showing the Distribution of Left ventricular dysfunction in the study population

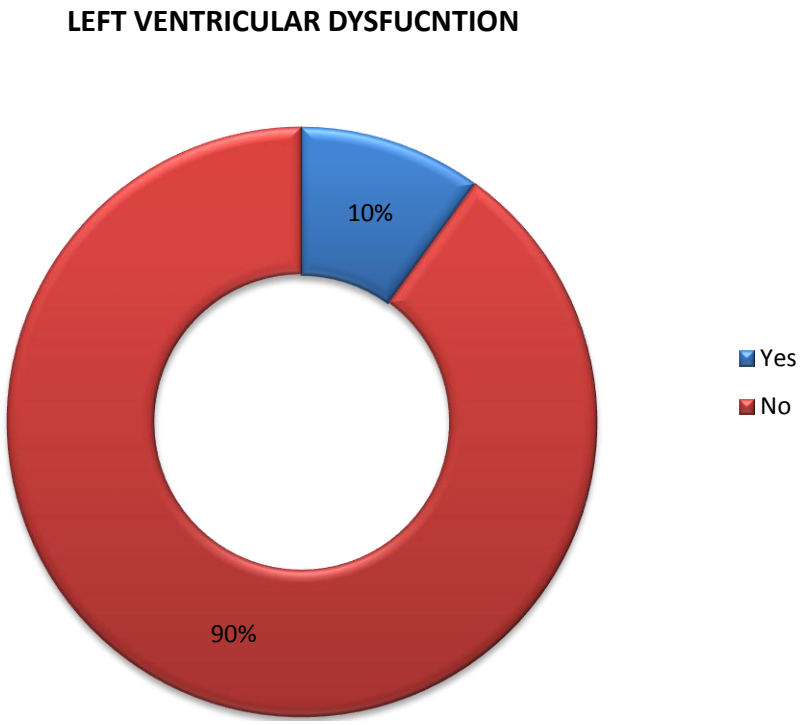

Fig. 17 LV DYSFUNCTION

Out of the 50 patients, 5 (10%) patients had Left Ventricular Dysfunction with ejection fraction (EF) less than 40.

DISTRIBUTION ACCORDING TO THE LEVEL OF EJECTION FRACTION (EF)

Table-2 Distribution according to the level of ejection fraction (EF)

EF	FREQUENCY	PERCENTAGE
LESS THAN 50	18	36
50-70	32	64
70	0	0
TOTAL	50	100

In the study, 36% of the patient had EF less than 50 and 64% had EF in the range between 50-70.

Low EF < 50 was associated with high FGF 23 (p value 0.025) and low EF <50 was associated with hypocalcemia (p value 0.013).

CORRELATION BETWEEN EJECTION FRACTION (EF) AND FGF23

In this study it was found that there was a negative correlation (r = -0.647) between EF and FGF23 and also the correlation was very highly significant ($p<0.001$).

Pie chart showing the Distribution of Hemodialysis Frequency in the study population

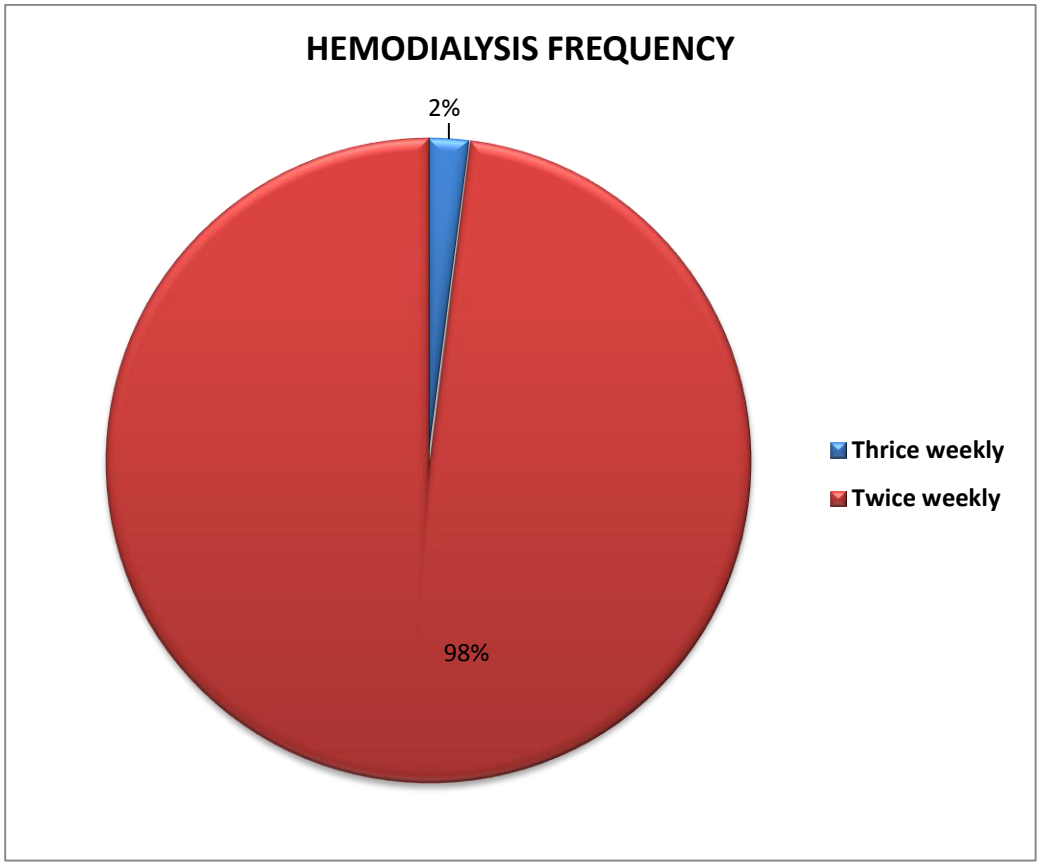

Fig 18. Hemodialysis Frequency

Among the 50 study participants, 98% of the study participants were undergoing twice weekly hemodialysis and 2% were on thrice weekly hemodialysis.

CALCIUM

Table -3 Distribution of Calcium Status among study subjects

CALCIUM STATUS	Frequency	Percentage
Hypocalcemia	32	64
Normal	18	36
Total	50	100

Among these patients, majority of them had hypocalcemia (64%). Mean calcium level was 8.172 mg/dl and a standard deviation of .873mg/dl.

PHOSPHATE

Pie chart showing the status of phosphate in the study population

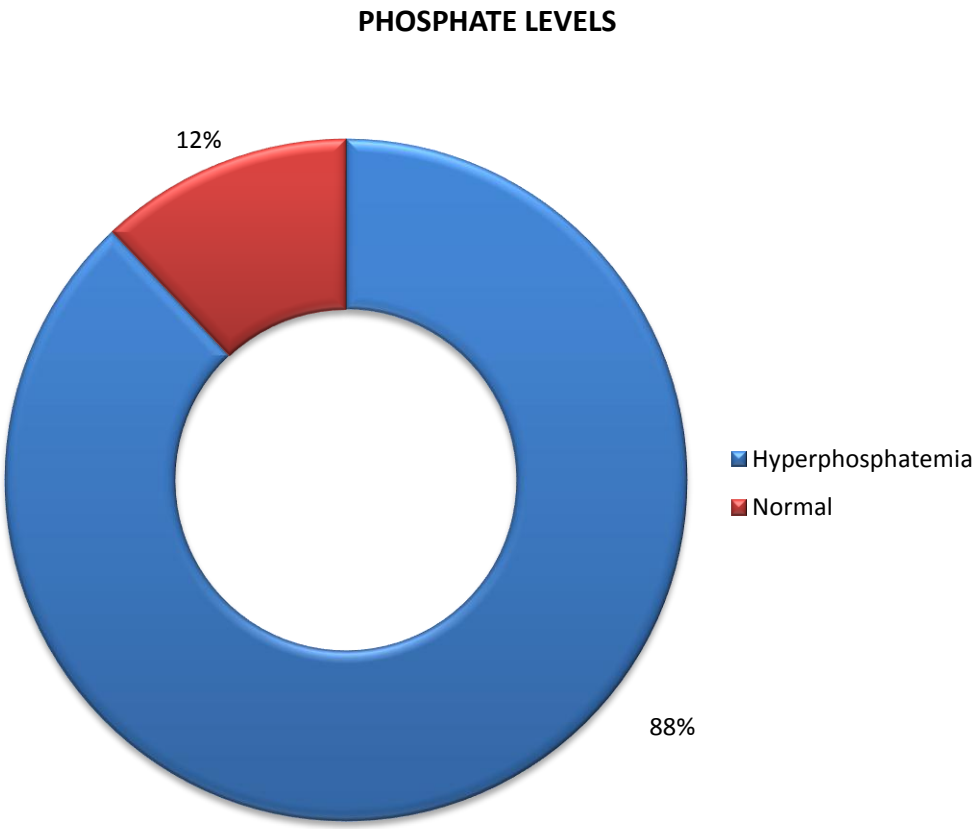

Fig 19 Status of phosphate in the study population

Mean phosphate level was 6.91mg/dl with a standard deviation of 1.978 mg/dl.

PARATHYROID HORMONE

Bar Chart showing the status of intact Parathyroid hormone(iPTH) in the study population

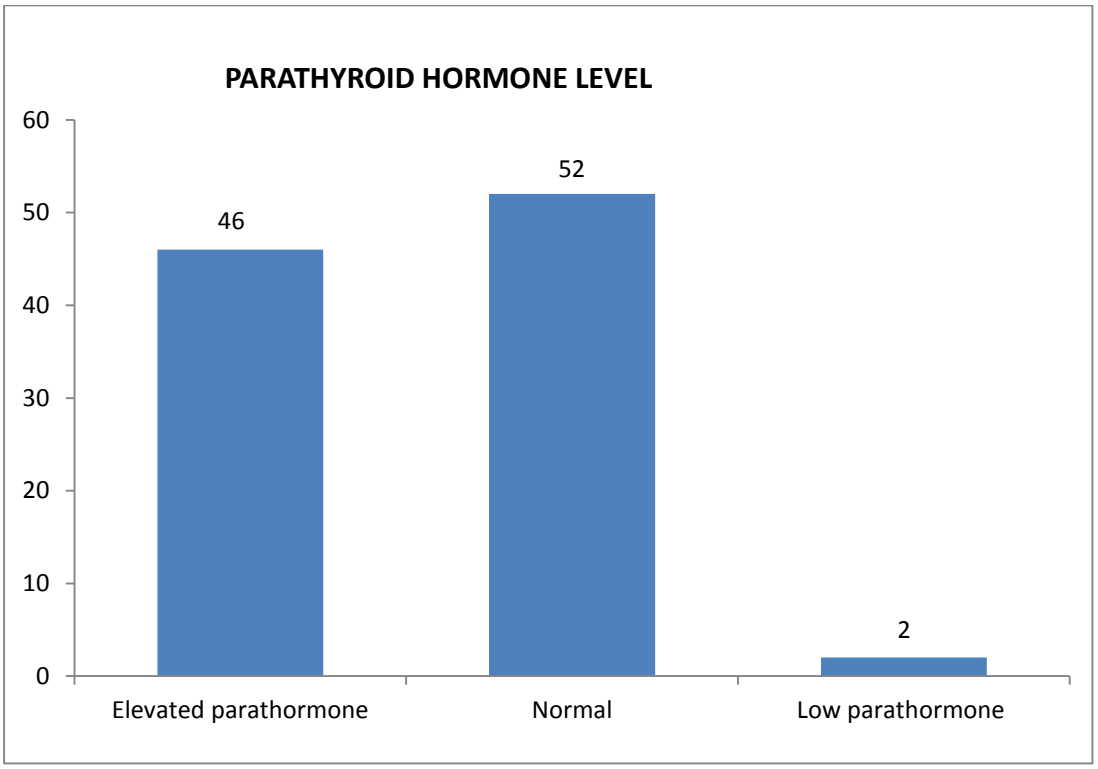

Fig 20. Parathyroid hormone level

Elevation of Parathormone levels were prevalent in study subjects (46%). Mean level parathyroid hormone level was 93.2pg/dl and standard deviation of 43.8

FIBROBLAST GROWTH FACTOR 23 (FGF23)

Distribution of FGF23 in the study population ranges from 19 to 1836.86.

Mean FGF23 was 279.57pg/ml and a standard deviation of 347.088pg/ml.

Figure showing the status of Fibroblast growth factor 23(FGF23) in the study population

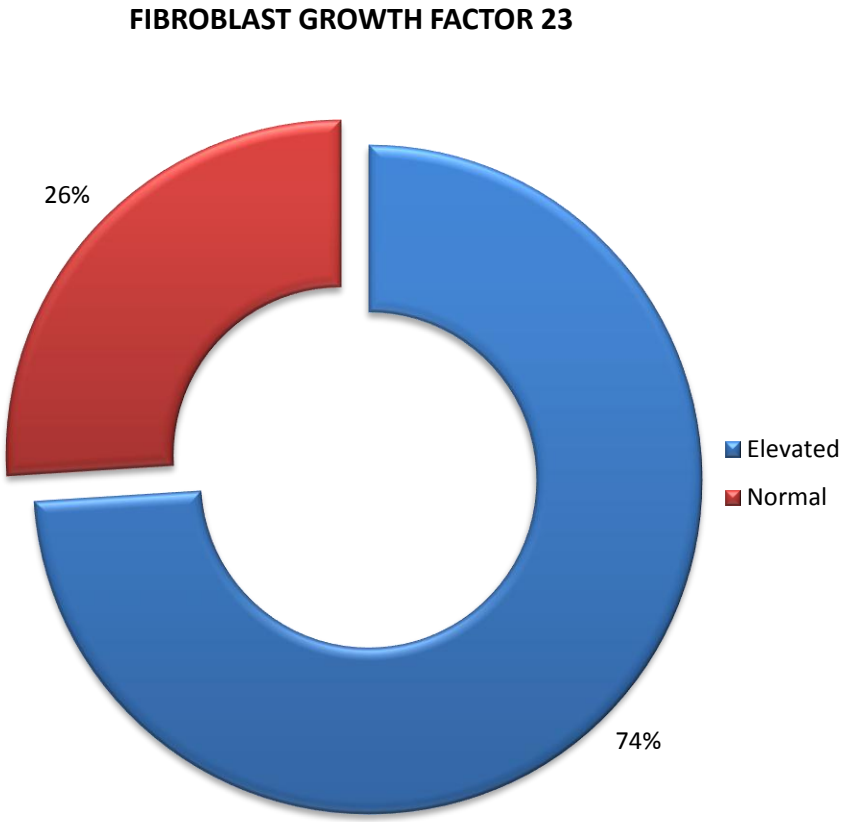

Fig. 21. Status of Fibroblast growth factor [FGF23] in study population

Among the 50 CKD subjects majority of them had elevated FGF23 levels (74%).

Table 4 Association between FGF23 and across different levels of phosphate

Phosphate	FGF23		Total
	Elevated N (%)	Normal N (%)	
Normal	1 (16.7)	5 (83.3)	6
Hyperphosphatemia	36(81.8)	8 (18.2)	44
Total	37	13	50

p=0.003

According to the above table, association between FGF23 and phosphate with p value of 0.003 was significant.

In this study, FGF23 with calcium was analysed. But no significant correlation of the levels of FGF23 and calcium (p value = 0.098) was seen.

Table 5 Relation between LVH with FGF23

Left Ventricular Hypertrophy (LVH) with Fibroblast growth factor (FGF23)

LVH	FGF23		Total
	Elevated N (%)	Normal N (%)	
Yes	10 (100)	0 (0)	10
No	27 (67.5)	13 (32.5)	40
Total	37	13	50

p=0.046*

Among 50 participants, 10 had left ventricular hypertrophy and FGF23 level was elevated in all left ventricular hypertrophy patients and the association was statistically significant ($p < 0.05$)

In the study, correlation between FGF23 and with other parameters like iPTH, hemoglobin, urea did not show significant correlation.

Table 6

Association between LVD and FGF23

LVD	FGF23		Total
	Elevated N (%)	Normal N (%)	
Yes	5 (100)	0 (0)	5
No	32 (71.1)	13 (28.9)	45
Total	37	13	50

P = 0.309

5 Participants had left ventricular dysfunction and FGF23 was elevated in all subjects and but there was no statistically significant association between them.

Correlation between phosphorus and urea

In this study, there was positive correlation (r =.376) between phosphorus and urea and correlation was highly significant $p < 0.01$.

Correlation between phosphorus and creatinine

Here there was positive correlation (r=.299) between phosphorus and creatinine also the correlation was statistically significant $p < 0.05$.

No significant correlation between iPTH and calcium in the study (with p value of 0.364).

No significant correlation between iPTH and phosphorous (p value of 0.281). Also there was no significant correlation between iPTH and hemoglobin in our study with a (p value 0.543)

In our study we tried to find correlation between haemoglobin and LVH (p value 0.363) and hemoglobin with LVD (p value of 0.192). There was no significant correlations

Table 7

Distribution of Biochemical Parameters and EF in the study population

Variable	Mean	SD	Minimum	Maximum
Urea	126.90	29.104	68	192
Creatinine	10.874	3.393	4.2	18.8
Calcium	8.172	0.873	6	9.6
Phosphorus	6.91	1.978	2.5	11.7
ALP	140.48	40.010	84	230
iPTH	93.238	48.885	12.2	256.6
Hb	8.67	1.888	4.6	14.6
EF	51.54	9.515	27	66
FGF 23	279.57	347.088	19	1836.86

Conclusion

DISCUSSION

The prevalence of CKD in the state of Tamil Nadu specifically in the Kanyakumari district has been found to be on the increase. The number of patients on renal replacement therapy has also been on the rise.

In this study, 50 patients were selected after taking informed consent. All of them were in the CKD stage 5. Though the present study was done, to find out the status of mineral bone disease in patients with CKD irrespective of the different stages of CKD. Importance was given to FGF23, along with other parameters like phosphorous, calcium and iPTH. FGF23 was selected with the intention of identifying an early biomarker for mineral bone disease in the patients selected for the study. This was also used to study relationship with the cardiovascular system.

At present, no Indian studies have shown its relevance in the status of CKD MBD. The previous studies already done, have not been able to strongly correlate FGF23 with the various parameters like calcium, phosphorous, parathormone etc.

The age group of study patients ranged from 20 years to 70 years. Here more than 50% were in the age group of 40 – 59 years. In these 80**%** were of the male gender and essentially the bread winners of the family.

All the patients taken up for the study were uniform in the etiology of CKD and had both diabetes and hypertension. Taking into account the duration of hypertension in these patients, out of 50, 5, 19 and 26 patients had hypertension for 1 year, up to 5 years and more than 5 years respectively. Similarly 23 subjects were

found to have DM for less than 5 years while 27 subjects were found to have DM for more than 5 years.

In these patients, 28**%** of them had previous history of cerebrovascular accidents. It was also seen that 32**%** had peripheral vascular disease. There was no correlation between gender and cerebrovascular accidents and peripheral vascular disease. This was done to note any disease of major blood vessels as CKD patients are at risk for the same.

Our study shows that these patients are at high risk for CVA and POVD. This correlates with other studies viz, Iseki.k, Fukiyama, k[140] found that the risk of stroke was five times higher in CKD patients than general population. In the current study 16 (32%) of study subject had PVD. In our study we found that FGF23 level was increased in all study subjects who were having PVD. Similarly Majad A.I. Mirza et 141 al found that increase in FGF23 has increased thickness of arterial wall which lead to PVD. Highlights of our study showed correlation of CVA around (28%) in our patients as well.

Study of FGF23 is a relatively new biomarker in the early detection of CKD MBD. It had been used to study and correlate the involvement in mineral bone disease in CKD patients. So far its correlation with different stages of kidney damages have not been strong.

Julia J et al [142] found that increases in FGF23 was associated with earliest abnormality of mineral metabolism in CKD patients. Similarly this study, showed 64% had hypocalcaemia. Significant association was found between increased levels of FGF 23 and hypocalcaemia which indicates mineral bone disease in this study group.

The distribution of haemoglobin and various biochemical parameters were observed along with FGF23. The levels of FGF23 ranged from 19 to 1836.86pg/ml with mean FGF23 was 279.57pg/ml and a standard deviation of 347.088pg/ml. Only two patients had values more than 1000pg/ml and 2 above 500pg/ml

One patient had phosphorus 11.7mg/dl, with serum calcium was 8mg/dl and iPTH was 146pg/ml. It is known that only when compensatory mechanisms to prevent phosphorous elevation fails, that phosphorous starts to rise. Here FGF23 was low and its value was 123pg/ml.

The lowest recorded FGF23 was 19pg/ml. This patient had phosphorous of 3.6mg/dl and calcium 6.6mg/dl. The highest level of FGF23 1836.86pg/ml was noted. This patient had phosphorous of 3.3mg/dl and calcium 8.8mg/dl.

Mean serum phosphorous level was 6.91mg/dl with a standard deviation of 1.978 mg/dl. It ranged from 2.5 to 11.7mg/dl.

There was positive correlation with elevated FGF23 and elevated phosphorous with p value of 0.003. The highest phosphorous noted was 11.7mg/dl. But this did not hold true when different ranges of phosphorous was considered. This shows that probably FGF23 increases only with increase in phosphorous. Mean serum phosphorous level was 6.91mg/dl with a standard deviation of 1.978 mg/dl. It ranged from 2.5 to 11.7mg/dl. The correlation was statistically significant when phosphorous levels were high.

Serum calcium levels had shown mean level of 8.172 mg/dl with standard deviation of .873mg/dl. It ranged from 6mg/dl to 9.6mg/dl. In this 32 (64%) had hypocalcaemia, 18 (36%) had normocalcaemia. No patient had hypercalcaemia. .

Hypocalcemia levels were as low as 6mg/dl. There was no correlation with low calcium level less than 8.4mg/dl and FGF23 (p value 0.098). Hence in this study there was no correlation of FGF23 with hypocalcemia.

The iPTH value ranged from 12.2pg/ml to 256.6pg/ml with mean of 93.238 - 48.885pg/ml. The levels of FGF23 did not correlate with the levels of iPTH either.

The blood urea level and serum creatinine levels ranged from 68mg/dl to 193mg/dl and 4.2mg/dl to 18.8mg/dl respectively. The means values were 126.9mg/dl with standard deviation of 29.104mg/dl for urea, and mean of 10.874mg/dl with standard deviation of 3.393mg/dl for creatinine was observed in these patients.

Haemoglobin ranged from 4.6gm% to 14.6gm% with mean of 8.67+/- 1.888gm%. The association with FGF23 was studied in particular. There was no correlation with the level of Haemoglobin.

To study the relationship between FGF23 and cardiovascular disease, assessment was done using left ventricular hypertrophy and left ventricular systolic dysfunction.

The number of patients having LVH were 10 (20%). LVH did not have correlation with age, gender, or vintage of dialysis. The LVH varied with different levels of FGF 23.

10 out of 50 patients had LV dysfunction. Their EF varied from minimum of 27 and maximum of 66. In the study low EF, had shown significant association with high FGF23 with p value of 0.025. This correlated with different levels of FGF23.

Thus the FGF23 was positively associated with LVH and different levels of ejection fraction (EF).

The present study shows that among the 50 participants, 10 (20%) had left ventricular hypertrophy and FGF23 level was elevated in all patients with left ventricular hypertrophy. This is similar to study by Ansel Philip Amaral et al[143] The FGF23 levels and rates of LVH were elevated in CKD and that elevated FGF23 was independently associated with LVH.

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LIMITATIONS

LIMITATIONS OF STUDY

1. This is small population and hospital based study
2. It is an expensive study as FGF23 is not routinely done other than for research purpose.
3. Patient included were only CKD 5 HD
4. Phosphorous has been shown to have diurnal variation and levels could vary
5. Phosphorous can vary in the same person depending on the diet and meal to meal variation is known
6. Confirmation of diagnosis of MBD is possible only with invasive study like bone biopsy
7. Levels of Vitamin D level was not evaluated

DISCUSSION

CONCLUSION

- CKD mineral bone disorder varies from patient to patient.
- Regular monitoring of calcium, phosphorus, iPTH is required.
- Individualised therapy is essential in the management of CKD - MBD.
- FGF23 can be utilised as a biomarker not only for identifying bone disease but also for cardiovascular disease as well.
- Recommended that measurements of FGF23 may be used for regular monitoring of CKD. With regular monitoring of all patients with CKD, the cost of the investigation should come down and long term expenditure incurred on the patients can be brought down.

SUMMARY

SUMMARY

CKD is a progressive disorder with involvement of various organs. CKD - MBD is a systemic disorder of mineral and bone metabolism which is manifested by either one or a combination of abnormalities of calcium, phosphorous, PTH or Vitamin D metabolism. This results in changes in bone mineralisation or growth and complications like vascular or other soft tissue calcification. The hormone fibroblast growth factor (FGF23) is derived from bone and its important function is in controlling phosphorous levels.

Elevation of serum levels of FGF23 is one of the possible causes of left ventricular hypertrophy and diastolic dysfunction. This leads to various morbidities and early mortalities in these patients.

This study was done in CKD patients to observe calcium, phosphorous, iPTH and especially FGF23 and its role in CKD MBD and cardiovascular involvement in them.

In our study, of the 50 study subjects 88 percent patients had hyperphosphataemia, 64 percent had hypocalcemia, 46 percent had elevated parathormone and 74 percent had elevated FGF23.

All the patients had biochemical evidence of mineral bone disorder. In them, 20 percent had left ventricular hypertrophy, and left ventricular diastolic dysfunction. Positive correlation was noted with levels of phosphorous and FGF23.

The left ventricular hypertrophy was also noted in those with elevated FGF23 and it was statistically significant. Statistically there was no correlation with the level of calcium, iPTH or reduced ejection fraction (left ventricular systolic function) in relation of FGF23.

BIBLIOGRAPHY

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APPENDIX

APPENDIX - 1

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES

KULASEKHARAM

RESEARCH COMMITTEE

CERTIFICATE

This is to certify that The Research Protocol Submitted by Dr. RISHABH GUPTA

Faculty / Post Graduate from Department of GENERAL MEDICINE

Titled To STUDY MINERAL BONE DISEASE & Cardiovascular morbidity in Chronic Kidney disease patients in relation with fibroblast growth factor 23(FGF-23) presenting to SMIMS.

is approved by the Research Committee.

Chair Person

Prof. & H.O.D.
Dept. of Bio-Chemistry
Sree Mookambika Institute of Medical Sciences
Kulasekharam 629 161

Convenor

Prof. & H.O.D.
Dept. of Physiology
Sree Mookambika Institute of Medical Sciences
Kulasekharam 629 161

Date :

APPENDIX - 2

INSTITUTIONAL HUMAN ETHICS COMMITTEE

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES,
KULASEKHARAM, TAMILNADU

Communication of Decision of the Institutional Human Ethics Committee(IHEC)

SMIMS/IHEC No:1 /Protocol no: 38 / 2016

Protocol title: STUDY OF MINERAL BONE DISEASE AND CARDIOVASCULAR MORBIDITY IN RELATION WITH FIBROBLAST GROWTH FACTOR 23 (FGF23) IN CHRONIC KIDNEY DISEASE PATIENT PRESENTING TO SREE MOOKAMBIKA INSTITUTE OF MEDICAL SEIENCES
Principal Investigator: Dr.Rishabh Gupta
Name& Address of Institution: Department of General Medicine Sree Mookambika Institute of Medical Sciences, Kulasekharam ☑ New review ☐ Revised review ☐ Expedited review
Date of review (D/M/Y): 15.12.2016 Date of previous review , if revised application:
Decision of the IHEC: ☑ Recommended ☐ Recommended with suggestions ☐ Revision ☐ Rejected
Suggestions/ Reasons/ Remarks:
Recommended for a period of : eighteen months

Please note*

- Inform IHEC immediately in case of any Adverse events and Serious adverse events.
- Inform IHEC in case of any change of study procedure, site and investigator
- This permission is only for period mentioned above. Annual report to be submitted to IHEC.
- Members of IHEC have right to monitor the trial with prior intimation.

Signature of Member Secretary IHEC

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES KULASEKHARAM IHEC Date 3/11/16

APPENDIX - 3

CASE RECORD FORM

OPD/IPD No. : Date:

Gender: Age:

Marital Status: Occupation:

Address:

Chief Complaints:

Past History

- DM - Yes ☐ No ☐
- HTN - Yes ☐ No ☐
- Renal Dysfunction - Yes ☐ No ☐

Family History:

General Examination

1) Pallor Present/ Absent
2) Pedal Edema Present/ Absent
3) Icterus Present/ Absent
4) Cyanosis Present/ Absent
5) Clubbing Present/ Absent
6) Lymphadenopathy Present/ Absent
7) Pedal edema Present/ Absent

8) JVP Present/ Absent

Height : …….. cm Wt : …….kg BMI …….kg/m ^{2}

Pulse: ……..bpm BP: ……...mmhg

BASE LINE INVESTIGATION

- Hb :
- RBS :
- RFT : Blood Urea Serum creatinine
- FGF 23 (pg/ml)
- S. Calcium (mg/dl)
- S. Phosphorus (mg/dl)
- iPTH(pg/ml)

o Parameters

- LVID (es)
- EF(%)
- RWMA

APPENDIX – 4

MASTER CHART

	Mr.										
S.No	Patient Name	Age	Sex	DM	HTN	PVD	CVA	Dialysis Vintage	Frequency of HD	Urea	Creatinine
1	Mr.Sunder Singh	58	Male	11 years	10 years	NO	NO	1 year	twice weekly	150	13.7
2	Mr. Neelakandan	36	Male	5 years	10 years	NO	NO	2 years	twice weekly	139	10.1
3	Mr.Masilaamani	68	Male	10 years	10 years	NO	NO	4 years	twice weekly	150	14.2
4	Mrs. Nazeema beevi	45	Female	12 years	8 years	NO	YES	4 years	twice weekly	81	8
5	Mr. Robin	50	Male	18 years	18 years	YES	NO	1 year	twice weekly	107	9
6	Mr.Sam sudin	63	Male	10 years	10 years	NO	YES	1 year	twice weekly	146	13.9
7	Mrs. Stella Rani	53	Female	2 years	10 years	YES	NO	1 year	twice weekly	115	8.3
8	Mr Pichandi pillai	66	Male	2 years	5 years	NO	NO	4 years	twice weekly	148	6.9
9	Mr.Ganesan	64	Male	5 years	3 years	YES	NO	3 years	twice weekly	95	8.5
10	Mr. Thomas Kurian	58	Male	16 years	9 years	NO	YES	7 years	twice weekly	146	12.7
11	Mrs.Mubeena beevi	61	Female	7 years	10 years	NO	NO	5 years	twice weekly	99	10.4
12	Mr.Ashok kumar	46	Male	3 years	5 years	NO	NO	4 months	twice weekly	80	16.2
13	Mr. Thavamani	51	Male	16 years	4 years	YES	YES	4 years	twice weekly	163	10.4
14	Mr.Vijayan	46	Male	5 years	3 years	NO	YES	3 months	twice weekly	119	16.3
15	Mr.Subbayan	46	Male	15 years	3 years	NO	NO	2 years	twice weekly	110	9.7
16	Mr.Maria john	49	Male	10 years	10 years	YES	NO	1 year	twice weekly	174	12.4
17	Mr.Mani	58	Male	3 years	3 years	NO	YES	2 years	twice weekly	115	8.8
18	Mr. Raymonds	43	Male	3 years	6 years	NO	NO	4 years	twice weekly	145	11.5
19	Mr. Chellathurai	41	Male	7 years	3 years	YES	NO	6 months	twice weekly	122	12.5
20	Mr. Vijayan	30	Male	9 years	3 years	NO	NO	3 years	twice weekly	166	18.8
21	Mr. Dennison	49	Male	2 years	7 years	NO	YES	3 years	twice weekly	143	12.1
22	Mr. Abdul Wahab	56	Male	5 years	4 years	NO	NO	4 years	twice weekly	145	17.8
23	Mr.Saji	36	Male	16 years	1 year	YES	NO	1 year	thrice weekly	76	7.9
24	Mr. Kumar	41	Male	4 years	5 years	YES	NO	1 year	twice weekly	149	6.9
25	Mrs. Pushpakumari	56	Female	5 years	10 years	YES	YES	2 years	twice weekly	157	11.5

26	Mr.Velayutham	49	Male	4 years	7 years	NO	NO	1.5 years	twice weekly	127	7.2
27	Mr. Prabhakaran	63	Male	18 years	7 years	NO	NO	1 year	twice weekly	144	12.4
28	Mr.Philiphose	70	Male	7 years	5 years	NO	NO	5 years	twice weekly	192	10.9
29	Mr Suresh	48	Male	16 years	1 year	NO	YES	1 year	twice weekly	120	7
30	Miss Koushal	20	Female	5 years	2 years	NO	NO	2 years	twice weekly	121	7.6
31	Mr Dhanapaul	47	Male	10 years	10 years	NO	NO	1 year	twice weekly	149	11.1
32	Miss Amudha	43	Female	2 years	25 years	YES	NO	1.5 years	twice weekly	146	9.5
33	Mr Albin jose	31	Male	7 years	3 years	YES	YES	3 years	twice weekly	141	16.8
34	Mrs Baby	47	Female	15 years	1 year	YES	NO	1 year	twice weekly	126	10
35	Mrs Shayilaja	49	Female	8 years	1 year	NO	NO	3 years	twice weekly	133	8.8
36	Mr Sekhar	55	Male	9 years	10 years	NO	NO	10 years	twice weekly	156	14.8
37	Mr David	60	Male	12 years	25 years	NO	NO	6 months	twice weekly	147	14.7
38	Mr Sathasiva Nair	70	Male	5 years	6 years	YES	YES	5 months	twice weekly	84	6.9
39	Mr Abraham	61	Male	5 years	2 years	NO	NO	4 montha	twice weekly	88	4.2
40	Mr Sundar raj	49	Male	8 years	6 years	NO	NO	11 months	twice weekly	118	9.4
41	Mr Nagappan	47	Male	5 years	3 years	NO	NO	4 years	twice weekly	144	15.2
42	Mrs. Vimala	69	Female	15 years	15 years	YES	NO	2 years	twice weekly	97	5.2
43	Mr Siva	29	Male	3 years	1 year	NO	YES	6 months	twice weekly	68	10.2
44	Mr George Vincent	51	Male	15 years	15 years	NO	NO	2 years	twice weekly	130	9.4
45	Mr Bala Chandran	48	Male	2 years	3 years	YES	NO	2.5 years	twice weekly	80	9.8
46	Mr Selvam	36	Male	5 years	2 years	NO	NO	4 years	twice weekly	154	14.4
47	Mrs. Margret Suganthi	60	Female	25 years	30 years	NO	NO	3 years	twice weekly	90	7.5
48	Mr.Maria Nesan	60	Male	8 years	4 years	YES	YES	3 years	twice weekly	101	6.2
49	Mr. Anish	25	Male	4 years	6 years	NO	YES	6 years	twice weekly	156	13.2
50	Mr Prabu	33	Male	5 years	8 years	NO	NO	3 years	twice weekly	93	12.8

Uric Acid	Ca	P	ALP	iPTH	Hb	BP	LVH	EF	PAH	LVD	FGF23 levels	No.of Hospilization
8.1	8.4	6.4	152	256.6	7.6	180/80	NO	64	NO	NO	120	5
3.3	8.1	8.1	194	46.1	10.5	180/100	NO	59	NO	NO	300	6
6.9	9.4	10	98	56.6	9	170/100	YES	39	YES	YES	925.57	6
6.1	9.3	10	111	48.3	7.9	210/100	NO	56	NO	NO	137.59	3
8.3	7.8	6.6	110	59.5	11.7	190/100	NO	50	NO	NO	141.8	4
3	8.2	6.8	134	102.3	9.2	180/80	NO	62	NO	NO	147	5
6.9	9.2	7.3	150	105.6	9.7	170/80	NO	48	NO	NO	201.45	6
5.6	8.2	6.1	98	45	8.3	190/100	NO	52	NO	NO	121.57	2
6.1	7.6	6.3	116	108.7	8.5	130/70	NO	56	NO	NO	130.47	8
7	8	9.2	96	59.3	7.8	190/100	YES	44	NO	NO	875	1
8.2	6.9	6.9	160	75.4	13.7	150/70	NO	66	NO	NO	198.57	9
7.1	8.9	5.5	93	56	8	180/80	NO	65	NO	NO	110.63	3
5.8	7.9	8	98	51.9	8.2	170/100	YES	53	NO	NO	327.75	14
3.8	8.7	8	86	61.5	7.9	180/100	NO	63	NO	NO	109.33	3
8.3	8.1	5.4	178	105	8.1	160/80	NO	66	NO	NO	200	6
8.9	8.4	7	200	85.1	7.5	150/70	NO	45	NO	NO	129.21	8
6.6	7.4	6	181	165.1	9.1	200/100	YES	32	NO	NO	435.49	8
2.6	7.7	8.2	156	45.9	7.4	160/100	NO	55	NO	NO	110.5	7
7.9	6.2	5.5	124	100	8.7	170/110	YES	34	YES	YES	1101	9
7.9	7.7	10.4	151	151.1	6.1	190/100	NO	56	NO	NO	187.45	5
8	8.2	6.6	194	121	10.4	180/90	NO	60	NO	NO	183.47	8
10	8.4	6.6	165	65.9	14.6	170/100	YES	51	YES	YES	509.16	7
3.7	9	6.4	178	100.14	8.2	160/50	NO	49	NO	NO	324.87	9
8.3	6.8	8.8	156	85.1	7.8	150/90	YES	61	NO	NO	392.2	6
6.4	9	7.8	135	45.3	8.4	140/100	NO	47	NO	NO	384.66	8
7.8	9.1	8.3	198	165	5	160/110	NO	39	NO	NO	233.23	6
6.2	9.1	8.3	154	148	10.3	180/100	NO	50	NO	NO	185.38	8
8.2	8.9	7.1	132	141.6	10.6	180/70	NO	48	NO	NO	256.15	9
2	9.5	6.7	200	191.1	8.2	160/90	NO	56	NO	NO	147.99	3

8.1	9	7.8	224	25.7	6.9	170/100	NO	44	NO	NO	212.23	7
6.8	7.8	5.8	220	85.8	9.7	180/60	YES	30	YES	YES	1253.84	6
7.4	7	7.3	230	74	4.6	150/90	NO	53	NO	NO	377.15	8
8.1	8.9	10.9	156	94	6.9	160/80	NO	43	NO	NO	202.38	3
6.8	9	8.4	89	65.1	7.1	150/50	NO	49	NO	NO	200.23	8
5.9	9.6	7.4	84	100.1	7.1	170/90	YES	55	NO	NO	323	7
9.9	6	7.3	147	151	12.3	180/110	NO	63	NO	NO	100.4	9
4.1	7.4	8.2	111	121.3	7.8	170/50	NO	49	NO	NO	94.69	5
5.3	8.1	4.1	156	45.4	7.4	160/100	NO	61	NO	NO	24	6
7	8.8	3.3	123	135.3	9.9	180/80	YES	27	YES	YES	1836.86	7
9.7	8.9	2.5	123	86.8	8.4	180/130	NO	55	NO	NO	29.65	9
7.4	8	11.7	165	146	11.1	180/100	NO	60	NO	NO	120.33	4
6.5	8.1	8.4	130	24.3	7.4	170/100	NO	52	NO	NO	91.22	6
6.3	8.4	5.4	138	175.2	9	200/110	NO	59	NO	NO	53.45	10
5.9	8.2	4.9	99	100.6	7.9	180/90	NO	51	NO	NO	99.24	14
4.4	8.2	3.8	110	51	10	150/90	NO	50	NO	NO	92.4	18
6.3	7.2	4.7	96	86.8	10	170/110	NO	55	NO	NO	64.63	12
3.5	8.6	6.8	89	12.2	8.7	190/80	NO	34	NO	NO	57.22	16
6	8.2	5	88	120.2	7.1	110/70	NO	49	NO	NO	79	18
9.1	6.5	3.9	123	48.1	8.5	180/110	NO	53	NO	NO	20.1	19
4.7	6.6	3.6	125	64.9	7.3	180/100	NO	59	NO	NO	19	16